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(54) Title: VECTORS HAVING ENHANCED EXPRESSION, AND METHODS OF MAKING AND USES THEREOF

(57) Abstract

Disclosed and claimed are vectors having enhanced expression and methods for making and using them. Enhancement of expression is from substantially co–temporal expression of at least one first nucleic acid molecule and at least one second nucleic acid molecule. The second nucleic acid molecule encodes a transcription factor or a translation factor or a transcription factor and a translation factor. The contemporaneous expression can be from operably linking the first and second nucleic molecules to a single promoter, or from operably linking the first nucleic acid molecule to a first promoter and the second nucleic molecule to a second promoter wherein the first and second promoters function substantially contemporaneously. Thus, the first and second nucleic acid molecules can be at the same locus in the vector, or at different loci. The second nucleic acid molecule can encode: one transcription factor or more than one transcription factor; or one translation factor or more than one translation factor; or at least one transcription factor and at least one translation factor. The transcription factor can be from vaccinia H4L, D6, A7, G8R, A1L, A2L, H5R, or combinations thereof. The translation factor can be from a K3L open reading frame, an E3L open reading frame, a VAI RNA, an EBER RNA, a sigma 3 open reading frame, a TRBP open reading frame, or combinations thereof. The vector can be a poxvirus such as an attenuated poxvirus, e.g., NYVAC, or ALVAC.

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TITLE OF THE INVENTION

VECTORS HAVING ENHANCED EXPRESSION, AND METHODS OF MAKING AND USES THEREOF

RELATED APPLICATIONS

Reference is made to the concurrently filed application of Tartaglia et al., "Vectors Having Enhanced Expression, And Methods of Making and Uses Thereof", Serial No. 08/815,809, incorporated herein by reference. Reference is also made to the copending applications of Paoletti et al., USSN 08/417,210, 08/303,275, 08/709,209, 08/184,009 (incorporating by reference USSN 07/805,567, from which U.S. Patent No. 5,378,457 issued) and 08/521,016 and to U.S. Patents Nos. 5,378,457, 5,225,336, 5,453,364, 5,494,807, 5,505,941, and 5,110,587, all of which are incorporated herein by reference.

FIELD OF THE INVENTION

The present invention relates to enhanced vectors, and methods for making and using them. The vectors can have enhanced transcription or translation or enhanced transcription and translation and/or expression, e.g., enhanced transcription or translation or transcription and translation and/or expression from a nucleotide sequence of interest.

Several publications are referenced in this application. Full citation to these publications is found where cited or at the end of the specification, immediately preceding the claims or where the publication is mentioned; and each of these publications is hereby incorporated by reference. These publications relate to the state of the art to which the invention pertains; however, there is no admission that any of these publications is indeed prior art.

BACKGROUND OF THE INVENTION

DNA such as plasmids or naked DNA, and other vectors, such as viral vectors, e.g., vaccinia virus and more recently other poxviruses, have been used for the insertion and expression from foreign genes. The basic

technique of inserting foreign genes into live infectious poxvirus involves recombination between pox DNA sequences flanking a foreign genetic element in a donor plasmid and homologous sequences present donor plasmid and homologous sequences present in the rescuing poxvirus (Piccini et al., 1987). Recombinant poxviruses are constructed in steps known as in or analogous to methods in U.S. Patent Nos. 4,769,330, 4,772,848, 4,603,112, 5,505,941, and 5,494,807, incorporated herein by reference. A desire in vector development is attenuated vectors, e.g., for enhanced safety; for instance, so that the vector may be employed in an immunological or vaccine composition.

For instance, the NYVAC vector, derived by deletion of specific virulence and host-range genes from the Copenhagen strain of vaccinia (Tartaglia et al., 1992) has proven useful as a recombinant vector in eliciting a protective immune response against an expressed foreign antigen. Likewise, the ALVAC vector, a vaccine strain of canarypox virus, has also proven effective as a recombinant viral vaccine vector (Perkus et al., 1995). In non-avian hosts, both these vectors do not productively replicate (with some exceptions as to NYVAC). Since all poxviruses replicate in the cytoplasm and encode most, if not all of the proteins required for viral transcription (Moss 1990), appropriately engineered foreign coding sequences under the control of poxvirus promoters are transcribed and translated in the absence of productive viral replication.

It would be an improvement over the state of the art to provide enhanced vectors, e.g., vectors having enhanced transcription or translation or transcription and translation and/or expression, for instance such vectors which are attenuated; especially since attenuation may raise issues of expression levels and/or persistence, and it would be an advancement to address such issues.

OBJECTS AND SUMMARY OF THE INVENTION

Recent studies on vaccinia replication have revealed certain poxvirus-encoded functions which play a role in the regulation of viral transcription and translation (reviewed in Moss, 1990; Moss, 1992). Some of these vaccinia encoded functions (e.g., E3L, K3L, H4L, and combinations thereof) have now surprisingly been utilized to increase the levels and persistence of gene expression (e.g., foreign gene expression) in vectors (e.g., the NYVAC and ALVAC vectors); and, are exemplary of the inventive vectors and methods.

Objects of the present invention may include at least one of: providing a method for increasing transcription or translation or transcription and translation and/or expression from at least one nucleotide sequence of interest by a vector, such as a coding nucleotide sequence by a vector; a vector having enhanced transcription or translation or transcription and translation; providing a method for preparing a vector having enhanced transcription or translation or transcription and translation and/or expression; providing a method for enhancing transcription or translation or transcription and translation and/or expression from a vector; providing an improved vector, such as poxvirus vectors, e.g., improved NYVAC, ALVAC or TROVAC vectors; and, products therefrom.

The invention thus provides a vector for enhanced expression of at least one first nucleotide sequence. The vector is modified to comprise at least one second nucleotide sequence encoding a transcription factor or translation factor or a transcription factor and a translation factor. The vector also can be modified to comprise the first nucleotide sequence. There is substantially co-temporal or substantially contemporaneous expression from the first and second nucleotide sequences. The expression is in a cell having a particular phenotype, and preferably the expression of

the first and second nucleotide sequences is with respect to the phenotype of the cell. Thus, expression of the second nucleotide sequence enhances expression of the first nucleotide sequence by enhancing transcription or translation or transcription and translation.

The first nucleotide sequence can be operably linked to a first promoter and the second nucleotide sequence can be operably linked to a second promoter, and the first and second promoters are preferably functional substantially co-temporally or contemporaneously. Thus, the first and second nucleotide sequences can be at different loci within the vector. The first and second nucleotide sequences also can be at the same locus within the vector, using the first and second promoters; or, by the first nucleotide sequence and the second nucleotide sequence being operably linked to a promoter.

The transcription factor can be of poxvirus origin, e.g., from a vaccinia virus. The transcription factor can be from an open reading frame selected from the group consisting of H4L, D6, A7, G8R, A1L, A2L, H5R, and combinations thereof. The translation factor can effect inhibition of eIF-2α phosphorylation or inhibition of PKR phosphorylation or otherwise sequester dsRNA which actually increases the concentration required to activate PKR. The translation factor can be selected from the group consisting of: a K3L open reading frame, an E3L open reading frame, a viral associated RNA I (VAI), an EBER RNA, a sigma 3 open reading frame, a TRBP open reading frame, and combinations thereof.

The first nucleotide sequence can be exogenous, e.g., encoding an epitope of interest, a biological response modulator, a growth factor, a recognition sequence, a therapeutic gene, a fusion protein or combinations thereof.

The vector can be a recombinant virus, such as a poxvirus; for instance, an orthopoxvirus or an avipoxvirus, e.g., a vaccinia virus, a fowlpox virus, a

canarypox virus; preferably an attenuated virus such as an attenuated poxvirus, e.g., NYVAC, ALVAC, or TROVAC.

The invention further provides a method for preparing a an inventive vector comprising modifying the vector to comprise the at least one second nucleotide sequence. The method can also include modifying the vector so that it comprises at the at least one first nucleotide sequence. Preferably the vector is so modified that there is substantially co-temporal or contemporaneous expression of the first and second nucleotide sequences; and, more preferably, the vector is also so modified that the expression is with respect to the phenotype of the cell. The method can comprise operably linking the first nucleotide sequence to a first promoter and the second nucleotide sequence to a second promoter, wherein the first and second promoters are functional substantially co-temporally or contemporaneously. The method can also comprise operably linking the first and second nucleotide sequences to a promoter.

The invention further provides an immunological, vaccine or therapeutic composition comprising at least one inventive vector and a pharmaceutically acceptable carrier or diluent.

The invention even still further provides a method for generating an immunological or therapeutic response in a host (animal, human, vertebrate, mammal, etc.) comprising administering to the host at least one inventive composition.

The invention additionally provides a method for increasing expression of at least one first nucleotide sequence by a vector comprising the first nucleotide sequence. The method comprises modifying the vector to comprise at least one second nucleotide sequence encoding a transcription factor or a translation factor or a transcription factor and a translation factor. There is preferably substantially co-temporal or

contemporaneous expression of the first and second nucleotide sequences. Expression can be in a cell having a particular phenotype; and it is more preferred to have expression be with respect to the phenotype of the cell. Expression of the second nucleotide sequence enhances expression of the first nucleotide sequence by enhancing transcription or translation or transcription and translation. The method can additionally comprise modifying the vector to comprise the first nucleotide sequence of interest.

The invention in yet another embodiment provides a method for expressing at least one gene product *in vitro* comprising infecting, or transfecting, a suitable cell with at least one inventive vector. The products therefrom can be an immunogen or epitope of interest, which can be useful in formulating therapeutic, immunological or vaccine compositions; or, for generating antibodies such as monoclonal antibodies; or, in assays, kits, tests and the like, such as diagnostic compositions, e.g., for detection of antibodies.

Thus, the invention can provide compositions and methods for *in vitro* transcription or translation or transcription and translation and/or expression involving at least one inventive vector, e.g., methods for producing a gene product (which can be used as an immunogen or epitope in a therapeutic, immunological or vaccine composition, or in a diagnostic or detection kit, assay or method, e.g., to ascertain the presence or absence of antibodies, or to generate antibodies, such as monoclonal antibodies, e.g., for use in a diagnostic or detection kit, assay or method), and/or for *ex vivo* transcription or translation or transcription and translation and/or expression involving at least one inventive vector, e.g., methods for producing a gene product for stimulating cells for reinfusion into a host (e.g., animal, mammal, vertebrate, human).

Additionally, in a further embodiment the

invention provides a method for expressing at least one nucleotide sequence (e.g., the at least one first nucleotide sequence) *in vivo* comprising administering at least one inventivie vector to a host (human, animal, vertebrate, mammal, etc.). The nucleotide sequence can encode an immunogen or epitope of interest. The method can obtain antibodies. From generating antibodies one can generate monoclonal antibodies; or, antibodies are useful in assays, kits, tests or diagnostic compositions, e.g., for detection of antigens.

The invention can thus provide methods and compositions for *in vivo* transcription or translation or transcription and translation and/or expression involving the inventive vectors, e.g., administering at least one inventive vector or a composition comprising at least one inventive vector, for instance, therapeutic, immunological or vaccine compositions comprising at least one inventive vector and a suitable carrier or diluent (e.g., suitable for veterinary and human medicine).

These and other embodiments are disclosed or are obvious from and encompassed by, the following Detailed Description.

BRIEF DESCRIPTION OF THE FIGURES

The following Detailed Description, given by way of example, but not intended to limit the invention to specific embodiments described, may be understood in conjunction with the accompanying Figures, incorporated herein by reference, in which:

Fig. 1 shows the nucleotide sequence of the insert in vP1380 containing the mutagenized H4L orf and lacZ orf under the H6 promoter (SEQ ID NO:);

Fig. 2 shows the nucleotide sequence of the ALVAC C8 Insertion site containing the H6/H42 expression cassette (SEQ ID NO:);

Fig. 3 shows the nucleotide sequence of the ALVAC C6 insertion site containing the H6/K3L and E3L expression cassette (SEQ ID NO:);

Fig. 4 shows the DNA sequence of the coding region of FHV gB with modified T5NT motifs (SEQ ID NO:);

Fig. 5 shows the DNA sequence of the H6 promoted FHV gB donor plasmid pC3H6FHVB (SEQ ID NO:);

Figs. 6 and 7 show DNA and amino acid sequences (SEQ ID NOS:) of inserts in vCP1433 and vCP1452; and

Fig. 8 shows the DNA sequence (SEQ ID NO:) of K3L E3L in vCP1452.

DETAILED DESCRIPTION

U.S. Patent No. 5,494,807, to Paoletti et al., hereby incorporated herein by reference, relates to a modified recombinant virus having inactivated virus-encoded genetic functions so that the recombinant virus has attenuated virulence and enhanced safety. The viruses disclosed in Paoletti et al. can be poxviruses, e.g., a vaccinia virus or an avipox virus, such as fowlpox virus and canarypox virus, e.g., NYVAC, ALVAC and TROVAC. ALVAC was deposited under the terms of the Budapest Treaty with the American Type Culture Collection (ATCC), 12301 Parklawn Drive, Rockville, Maryland, 20852, USA, ATCC accession number VR-2547. TROVAC was likewise deposited under the terms of the Budapest Treaty with the ATCC, accession number 2553. And, NYVAC (vP866), vP994, vCP205, vCP1433, placZH6H4Lreverse, pMPC6H6K3E3 and pC3H6FHVB were also deposited with the ATCC under the terms of the Budapest Treaty, accession numbers _____, _____, _____, _____, _____, _____, and _____, respectively.

Like the Paoletti et al. issued U.S. Patent, Falkner et al., WO 95/30018, published November 9, 1995, based on U.S. application 08/235,392, filed April 24, 1994 (both incorporated herein by reference), relates to poxviruses wherein loci for genetic functions associated with virulence (i.e., loci for "essential" functions) are employed for insertion of exogenous DNA.

Further, recombinants can be made from early (DNA^-) and late defective mutants (<u>see</u> Condit and Niles,

"Orthopoxvirus Genetics," pp 1-39, In: Poxviruses, Edited by R. W. Moyer and P. C. Turner (Springer-Verlag, 1990), and documents cited therein, hereby incoporated herein by reference)), or from MVA which is said to be abortive late. Recombinants from defective mutants, abortive late viruses, viruses having essential genetic functions deleted or interrupted, or viruses having expression without productive replication (e.g., ALVAC in mammalian systems) may be said to be attenuated.

Certain vectors, such as attenuated vectors, e.g., NYVAC and ALVAC vectors, are blocked or limited in late gene expression in mammalian cells. Thus, early promoters are routinely employed in such vectors, e.g., NYVAC- or ALVAC-based recombinants, for expression from the foreign gene products.

Vaccinia encodes an open reading frame (ORF) designated H4L which has been shown to be required for early viral transcription (Ahn and Moss 1992, Zhang et al, 1994). The H4L ORF encodes an essential protein of 94 kDa which is expressed after the start of viral DNA replication (late function). The H4L protein has been found to be tightly associated with the viral RNA polymerase complex and is believed to act in conjunction with the vaccinia early transcription factor (VETF) to initiate and transcribe early viral message (Ahn and Moss, 1992).

H4L is expressed late, but required early. This is consistent with the protein being packaged in the viral particles similar to that which is observed with VETF. This suggested that the amount of H4L present at early times post infection is low and perhaps limiting. Hence, one approach to increase foreign gene expression in an abortive, early vector-, e.g., virus-host interaction would be to increase the amount of H4L protein available during the early phase by expressing the H4L ORF using a vaccinia early/late promoter rather than the endogenous late promoter. Early expression from

H4L may not only increase the level of foreign gene transcripts, but also increase levels of other vaccinia early genes (e.g. E3L) which may also increase total protein levels.

There are other viral transcription factors; for instance, early and/or late viral transcription factors of poxvirus origin; e.g., from: vaccinia D6, vaccinia A7, vaccinia G8R, vaccinia A1L, vaccinia A2L, or vaccinia H5R (VLTF-1, -2, -3, -4, P3, VLTF-X; see Kovacs et al., J. Virology, October 1996, 70(10):6796-6802, and documents cited therein, incorporated herein by reference). These and other transcription factors, and nucleotide sequences therefor or for homologs thereof, e.g., from another poxvirus, are useful in the practice of the invention.

The selection of a suitable transcription factor is within the ambit of the skilled artisan from this disclosure and knowledge in the art; for instance, the skilled artisan can select a transcription factor based on an abortive phenotype of the vector, e.g., MVA is said to be abortive late, and a late or early or early/late transcription factor may be employed with this vector; ALVAC is abortive early and an early or early/late transcription factor may be employed with this vector; and, the vector can also be a ts (temperature sensitive) mutant (with respect to early (DNA^-) and late defective mutants which can be also used in the practice of this invention, reference is made to Condit and Niles, *supra*). Thus, it is preferred that the transcription and/or translation factor and the at least one nucleotide sequence of interest be expressed early, late (including intermediate), or early/late, relative to the phenotype of the vector.

Another means to increase foreign gene expression involves enhancing the overall efficiency of translation, e.g., mRNA translation, such as viral mRNA translation. Two vaccinia encoded functions (E3L and

K3L) have recently been identified as playing a role in the regulation of viral translation (Beattie et al., 1995a, 1995b, 1991; Chang et al., 1992; Davies et al., 1993). Both are capable of inhibiting the action of a cellular protein kinase (PKR) which, when activated by double stranded RNA (dsRNA), phosphorylates the translational initiation factor eIF-2α, leading to an inhibition of initiation of mRNA translation (reviewed in Jacobs and Langland, 1996). Vaccinia virus, which produces dsRNA during viral transcription, has thus evolved mechanisms to block the negative action of PKR on eIF-2α and allow for efficient translation of viral mRNA. (Asymetric transcription gives rise to dsRNA; any viral infection or plasmid derived expression gives rise to it; dsRNA activates PKR; PKR becomes autophosphorylated, leading to phosphorylation of eIF-2α.)

The vaccinia K3L ORF has been shown to have significant amino acid homology to eIF-2α (Goebel et al., 1990; Beattie et al., 1991; U.S. Patent No. 5,378,457; <u>see also</u> Beattie et al., 1995a, 1995b). This protein is believed to act as a pseudosubstrate for PKR and competes for the eIF-2α binding site (Carroll et al., 1993; Davies et al., 1992). The K3L gene product can bind to activated PKR and thus prevent phosphorylation of eIF-2α with its resultant negative effect on translation initiation.

The vaccinia E3L gene codes for a protein which is capable of specifically binding to dsRNA (Watson and Jacobs, 1991; Chang et al., 1992). This would tend to lower the amounts of dsRNA in the infected cell, and thus reduce the level of activated PKR. When E3L was deleted from vaccinia, the resulting virus lost this kinase inhibitory function and further allowed activation of the 2′ 5′ oligoadenylate synthetase/RNase L pathway resulting in increased degradation of rRNA (Beattie et al., 1995a, 1995b). Thus, E3L appears to be critical for efficient mRNA translation in vaccinia infected cells at two

levels; mRNA stability and limiting eIF-2α phosphorylation.

The ALVAC genome has been sequenced and searched for any homology to E3L/K3L or to any known dsRNA binding motif. Results have revealed no significant homology of any ALVAC ORFS to these two vaccinia ORFs, nor the presence of any dsRNA binding motifs.

Thus, an approach to improving expression levels in recombinant ALVAC vectors was to express the vaccinia E3L/K3L ORFs in ALVAC under the control of early vaccinia promoters. Through inhibition of PKR in the infected cells, the levels and persistence of foreign gene expression could be enhanced.

Hence, NYVAC and ALVAC recombinants as discussed herein were generated in order to enhance foreign gene expression at the transcriptional or translational or transcriptional and translational levels, as examples of the vectors and methods of the present invention.

Thus, exemplified herein is NYVAC recombinants having an early expressed H4L ORF and ALVAC recombinants having expression from the vaccinia E3L/K3L genes for enhancing or increasing the levels or persistence of expression of an inserted foreign gene. The up-regulation of foreign gene expression can have a profound effect on the induction of a therapeutic or immunological response in a host administered or inoculated with recombinants derived from these new vectors, thereby leading to an enhanced immunological, e.g., protective, response, or an enhanced therapeutic response.

The scope of the invention, i.e., to manipulate expression from any of a transcription and/or translation factor, e.g., H4L, E3L and K3L, to thereby enhance transcriptional or translational or transcriptional and translational and/or expression efficiency, can be extended to other eukaryotic vector systems (i.e. DNA,

viruses).

In fact, viruses in other families have also evolved mechanisms to overcome the cellular anti-viral response of translational down-regulation through PKR activation. In adenoviruses, the VAI RNA, transcribed by RNA pol III, has been well characterized and shown to bind directly to PKR, and thus, prevent its activation by dsRNA (Mathews and Shenk, 1991). Deletion of VAI from the adenovirus genome results in a mutant that replicates poorly and is deficient in levels of late gene expression (Thimmappaya et al., 1982). Similarly, Epstein-Barr virus, a herpesvirus, has an analogous RNA, called EBER, which also acts to prevent PKR activation by directly binding to the kinase (Clark et al., 1991; Sharp et al., 1993). The reovirus sigma 3 gene product has been shown to act in a similar manner as vaccinia E3L in binding dsRNA and thus preventing activation of PKR (Imani and Jacobs, 1988; see also Beattie et al. 1995a). Indeed, one study has shown that the reovirus sigma 3 gene can partially compensate a vaccinia recombinant deleted of E3L (Beattie et al., 1995a). Further, a cellular protein activated upon HIV infection (TRBP) has been shown to inhibit the activity of PKR (Park et al., 1994).

Thus, the present invention broadly relates to manipulation of expression, preferably by employing at least one transcription factor, e.g., at least one early and/or late viral transcription factor, or at least one translation factor, e.g., a nucleotide sequence encoding a product for overcoming the cellular anti-viral response of translational down-regulation through PKR activation in any eukaryotic vector system, or at least one trancription factor and at least one translation factor; for instance, to increase or enhance expression. And, the invention can pertain to any vector system, including, plasmid or naked DNA vectors, viral vectors, such as poxvirus, adenovirus, herpesvirus, baculovirus, and the like. Thus, the nucleotide sequences can be RNA

or DNA, for instance, as is suitable in view of the vector system.

Accordingly, the invention can relate to a vector modified to comprise at least one nucleotide sequence encoding at least one transcription factor, at least one translation factor, or at least one transcription factor and at least one translation factor; a method for increasing transcription and/or translation and/or expression by a vector or for preparing an inventive vector, e.g., by modifying the vector to comprise the at least one nucleotide sequence.

These methods can include substantially co-temporal expression from: (i) a first nucleotide sequence comprising at least one nucleotide sequence of interest, and (ii) a second nucleotide sequence comprising at least one nucleotide sequence encoding a transcription factor, or at least one nucleotide sequence encoding a translation factor or at least one nucleotide sequence encoding a transcription factor and a translation factor. The vector also can be modified to comprise the at least one nucleotide sequence of interest. The at least one nucleotide sequence of interest can be at least one coding nucleotide sequence. The vector preferably has substantially co-temporal or contemporaneous expression of the first and second nucleotide sequences.

The substantially co-temporal expression can occur by employing promoters for the first and second nucleotide sequences which are functional at approximately the same time or stage of infection. Thus, the nucleotide sequence of interest and the nucleotide sequences encoding the factor(s) can be positioned at different loci in the vector. Alternatively, substantially co-temporal expression can occur by positioning the first and second nucleotide sequences within the same loci. Thus, substantially co-temporal expression can occur by operably linking to the nucleotide sequence of interest and/or to a promoter

operably linked to the nucleotide sequence of interest, a nucleotide sequence encoding a transcription factor, a nucleotide sequence encoding a translation factor, or a nucleotide sequence encoding a transcription factor and a translation factor.

The transcription factor can be from any suitable system. Preferably, the transcription factor is of poxvirus origin, e.g., from a vaccinia virus. The transcription factor can be from expression from an open reading frame selected from the group consisting of H4L, D6, A7, G8R, A1L, A2L, H5R, a homolog thereof and combinations thereof. It is also preferred that embodiments including a nucleotide sequence encoding a transcription factor comprise a poxvirus vector system.

The translation factor can likewise be from any suitable system. Preferably the translation factor can effect inhibition of eIF-2α phosphorylation or inhibition of PKR phosphorylation or otherwise decreases cellular dsRNA content which increases the effective concentration of dsRNA. The translation factor can be selected from expression from the group consisting of: a K3L open reading frame, an E3L open reading frame, a VAI RNA, an EBER RNA, a sigma 3 open reading frame, a TRBP open reading frame, a homolog thereof, and combinations thereof. The term "effective" with respect to dsRNA concentration means the amount of dsRNA to activate PKR and/or eIF-2α phosphorylation (the dsRNA being in a form therefor). With respect to RNA-based factors, e.g., VAI RNA, EBER RNA, the skilled artisan can obtain suitable DNA from the RNA for use in a DNA vector system without undue experimentation. And, with respect to DNA-based factors, the skilled artisan can obtain suitable RNA therefrom for use in a RNA vector system, without undue experimentation.

The term "substantially co-temporal expression" or the term "substantially contemporaneous expression" means that the nucleotide sequence(s) encoding the

transcription or translation or transcription and translation factor(s) are expressed during approximately the same stage of infection as is the at least one nucleotide sequence of interest.

For instance, poxvirus genes are regulated in a temporal manner (Coupar, et al., Eur. J. Immunol., 1986, 16:1479-1487, at 1479). Thus, immediately after infection, a class of "early" genes is expressed (Id.). "Early genes" cease being expressed (i.e., early promoters cease functioning) at a time after infection prior to the "later" stage of infection (DNA replication commencement). The thymidine kinase ("TK") gene and TK promoter is an example of an immediate "early" gene and promoter (Hruby et al., J. Virol., 1982, 43(2):403-409, at 403). The TK gene is switched "off" about four hours after infection. "Late genes" are a class of genes not expressed until DNA replication has commenced (Coupar et al., *supra*). The PL11 promoter employed by Coupar et al. is an example of a "late" promoter. Thus, in Coupar et al., HA gene expression regulated by the PL11 promoter was not until after DNA replication, despite being in the TK region.

In contrast to canonical "early" genes and "late" genes the 7.5 kD gene and 7.5 kD promoter, is an example of an "early and late" gene and promoter. An "apparent exception to regulated transcription" (Davison and Moss, "Structure of Vaccinia Virus Early Promoters" J. Mol. Biol., 210-69, 249-69 (1989) at 749), the 7.5 kD promoter "contains regulatory signal for both early and late transcription" (Coupar et al., *supra*). Indeed, there are "independent early and late RNA start sites within the promoter region of the 7.5-kD gene" (Cochran et al., J. Virol., 59(1): 30-37 (April, 1985).

Coupar et al. observed "that temporal regulation of HA expression by the promoters PF [early], P7.5 [early and late] and PL11 [late] was maintained when the promoters were transposed to interrupt the TK gene of

[vaccinia virus]" (Id., at 1482). That is, Coupar et al. observed that foreign gene expression under the control of an early vaccinia promoter occurred "early", foreign gene expression under control of a late vaccinia promoter occurred "late", and foreign gene expression under the control of the early and late vaccinia 7.5 kD promoter occurred both early and late (See also id. at 1479: "[p]romoter sequences transposed to within the thymidine kinase (TK) gene continue to function in a temporally regulated manner" (citations omitted)).

Thus, the nucleotide sequence(s) encoding the transcription or translation or transcription and translation factor(s) can be under the control of a first type of promoter and the at least one nucleotide sequence of interest or the coding nucleotide sequence can be under the control of a second type of promoter, wherein the first and second promoters are both early, both late (including intermediate), or both early and late; or, the first promoter can be early or late and the second promoter early and late; or the first promoter can be early and late and the second promoter early or late. The nucleotide sequence of interest and the nucleotide sequence(s) encoding the transcription or translation or transcription and translation factor(s) can be at the same locus or at different loci; or under the control of the same promoter.

Accordingly, the invention can relate to a method for preparing a vector having enhanced transcription or translation or transcription and translation and/or expression, or to a method for increasing or enhancing transcription or translation or transcription and translation and/or expression in a vector comprising operably linking to at least one nucleotide sequence of interest, or to a promoter operably linked thereto, at least one nucleotide sequence for at least one transcription and/or at least one translation factor; e.g., at least one nucleotide

sequence for a transcription transcription factor, or at least one nucleotide sequence for a translation factor or at least one nucleotide sequence for a transcription factor and a translation factor. Preferably the translation factor effects an inhibition of eIF-2α phosphorylation and/or effects an inhibition of phosphorylation of PKR and/or a cellular kinase responsible for phosphorylation of eIF-2α and/or effects the effective concentration of dsRNA. The invention also can thus relate to vectors from such methods.

Alternatively, the inventive methods can comprise operably linking at least one nucleotide sequence of interest to a first type of promoter and operably linking at least one second nucleotide sequence encoding at least one transcription and/or translation factor to a second type of promoter, within a vector, wherein the first and second promoters are both functional at the same time or same stage of infection, e.g., the first and second promoters are both early, both late (including intermediate), or both early and late; or, the first promoter is early or late and the second promoter is early and late; or the first promoter is early and late and the second promoter is early or late. Of course, the first and second promoters can be the same promoter at two or more different loci, or the same promoter at one locus. And, the invention thus relates to vectors from such methods.

And, the term "nucleotide sequence" as used herein can mean nucleic acid molecule. Thus, a nucleotide sequence can be an isolated nucleic acid molecule, e.g., exogenous DNA.

Accordingly, the present invention can provide vectors modified to contain at least one exogenous nucleotide sequence, preferably encoding at least one epitope of interest, and at least one transcription factor or at least one translation factor or at least one transcription factor and at least one translation factor,

wherein there is substantially temporal co-expression (or substantially co-temporal expression or substantially contemporaneous expression) of the exogenous nucleotide sequence and the factor(s); and, methods for making and using such vectors and products therefrom. Enhanced or improved expression is obtained by the vectors and methods of the invention; and, enhanced or improved expression can mean enhanced levels and/or persistence of expression.

The invention can thus provide vectors, for instance, poxvirus vectors, which are abortive early, e.g., NYVAC, ALVAC or TROVAC recombinants, having an early expressed transcription factor, e.g., an early expressed H4L open reading frame (or a homolog thereof, e.g., from another vector system, such as poxviruses other than vaccinia, herpesvirus, such as Epstein-Barr, adenovirus, plasmid or naked DNA, and the like) as a means for enhancing and/or increasing the levels and/or persistence of an inserted nucleotide sequence, e.g., a foreign gene. The invention can also provide vectors, for instance, poxvirus vectors, which are abortive late (which includes abortive intermediate), e.g., MVA recombinants, having a late expressed transcription factor, e.g., an expressed G8R, A1L, A2L, H5R (VLTF-1, -2, -3, -4, P3, VLTF-X) open reading frame (or a homolog thereof, e.g., from another vector system, such as poxviruses other than vaccinia, herpesvirus, such as Epstein-Barr, adenovirus, plasmid or naked DNA, and the like) as a means for enhancing and/or increasing the levels and/or persistence of expression from an inserted nucleotide sequence, e.g., a foreign gene.

The invention can additionally provide vectors, for instance, poxvirus vectors, e.g., NYVAC, ALVAC or TROVAC recombinants, having expression from the vaccinia E3L and/or K3L (or a homolog thereof, e.g., from another vector system, such as poxviruses other than vaccinia, herpesvirus, such as Epstein-Barr, adenovirus,

plasmid or naked DNA, and the like, note discussion *supra* of viral mechanisms to overcome the cellular anti-viral response of translational down-regulation through PKR activation) as a means for enhancing and/or increasing the levels and persistence of an inserted nucleotide sequence, e.g., a foreign gene.

Even further still, the invention can provide vectors, for instance, poxvirus vectors, e.g., NYVAC, ALVAC or TROVAC recombinants, having an early expressed transcription factor, e.g., an early expressed H4L open reading frame (or a homolog thereof) and/or a late expressed transcription factor, e.g., an expressed G8R, A1L, A2L, H5R (VLTF-1, -2, -3, -4, P3, VLTF-X) (or a homolog thereof), for instance abortive late (which includes abortive intermediate), e.g., MVA, recombinants, and expression from the vaccinia E3L and/or K3L (or a homolog thereof) as a means for enhancing and/or increasing the levels and persistence of expression from an inserted nucleotide sequence, e.g., a foreign gene.

As shown in the Examples below, ALVAC-HIV recombinant vCP1452 containing the K3L/E3L factors had enhanced expression on human cells in comparison to vCP1433 or vCP300. Indeed, enhanced expression is observed with the E3L/K3L translational factors in human and canine cells.

Enhanced expression by translational factors such as E3L/K3L may be cell type dependent. For instance, while enhanced expression with E3L/K3L is observed in human and canine cells it is not observed in murine and feline cells. From this disclosure and the knowledge in the art, the skilled artisan can select an appropriate translational factor for use with a particular cell type, without undue experimentation. For example, it should go without saying that the skilled artisan knows the differences between cells. Thus it is preferred that the translational factor be expressed in a cell in which enhanced expression is observed, e.g., that

the translational factor employed be with respect to the cell.

Further, preliminary immunogenicity studies in mice show no evidence of enhanced immunogenicity by the E3L/K3L translational factor. This corresponds to no observed enhanced expression in murine cells. Accordingly, the skilled artisan from this disclosure and the knowledge in the art can select a translational factor which will provide enhanced immunogenicity in a desired animal, without undue experimentation. If enhanced expression is observed *in vitro* in a particular cell line by a particular translational factor, e.g., E3L/K3L in human or canine cells, the skilled artisan can thus expect enhanced immunogenicity *in vivo* in the animal (including human) from which the cells were derived by that particular translational factor, e.g., enhanced immunogenicity in humans and canines from the E3L/K3L translational factor.

Furthermore, in murine cells, the limiting factor of ALVAC expression is at the transcription level. Accordingly, use of an appropriate transcription factor can overcome the inability to observe enhanced expression in the murine system. Thus, the origin of the cell may be a factor in *in vitro* or *in vivo* applications of the invention (note H4 data), as may be the nature of the vector, e.g., the phenotype of the vector; but, appropriate selection of a cell and vector phenotype and of time of expression from factor(s) and foreign and/or exogenous DNA are within the ambit of the skilled artisan, from this disclosure and the knowledge in the art, without undue experimentation.

Also, the Examples below show that NYVAC recombinant vP1380 has enhanced expression levels in comparison to vP994. Possibly, part of the enhanced levels in vP1380 are due to enhanced transcription and expression from viral specific products such as E3L, such that there is enhanced transcription and translation

involved in expression in vP1380. There is more expression from the exogenous DNA and at more persistent levels in vP1380, in accordance with the invention wherein vectors obtain greater levels of expression and more persistent levels of expression.

Enhanced expression profiles in the murine system provided enhanced immunogenicity in mice, as shown by vP1380 being more immunogenic in mice than vP994. Another observation is that enhancement profiles are seen in restrictive early cells in the abortive early NYVAC recombinants herein, whereas the profiles were not observed in cells where there was productive replication, e.g., VERO or CEF, suggesting that it may be preferred that the factor and the foreign DNA be expressed substantially co-temporally or contemporaneously, i.e., that preferably there be co-expression at substantially the same time or stage, and that the time of expression, e.g., early, late, early and late, should be matched with the phenotype of the vector (e.g., abortive early, abortive late), i.e., that in a system in which viral replication is not impaired (a permissive system) or in a system in which replication is aborted at a time when expression is not matched with the phenotype of the vector may not obtain optimal expression. Thus, in an abortive early system such as ALVAC or NYVAC, one preferably expresses exogenous DNA and the transcriptional or translational or transcriptional and translational factor(s) early; in an abortive late system, one preferably expresses exogenous DNA and the transcriptional or translational or transcriptional and translational factor late or early and late (as expression only early may be akin to expression in a permissive system, i.e., one may not necessarily obtain optimal expression).

The methods for making a vector or recombinant can be by or analogous to the methods disclosed in U.S. Patent Nos. 4,603,112, 4,769,330, 5,174,993, 5,505,941,

5,338,683, 5,494,807, and 4,722,848, WO 95/30018, Paoletti, "Applications of pox virus vectors to vaccination: An update," PNAS USA 93:11349-11353, October 1996, Moss, "Genetically engineered poxviruses for recombinant gene expression, vaccination, and safety," PNAS USA 93:11341-11348, October 1996, Smith et al., U.S. Patent No. 4,745,051 (recombinant baculovirus), Richardson, C.D. (Editor), Methods in Molecular Biology 39, "Baculovirus Expression Protocols" (1995 Humana Press Inc.), Smith et al., "Production of Huma Beta Interferon in Insect Cells Infected with a Baculovirus Expression Vector," Molecular and Cellular Biology, Dec., 1983, Vol. 3, No. 12, p. 2156-2165; Pennock et al., "Strong and Regulated Expression of *Escherichia coli* B-Galactosidase in Infect Cells with a Baculovirus vector," Molecular and Cellular Biology Mar. 1984, Vol. 4, No. 3, p. 399-406; EPA 0 370 573, U.S. application Serial No. 920,197, filed October 16, 1986, EP Patent publication No. 265785, U.S. Patent No. 4,769,331 (recombinant herpesvirus), Roizman, "The function of herpes simplex virus genes: A primer for genetic engineering of novel vectors," PNAS USA 93:11307-11312, October 1996, Andreansky et al., "The application of genetically engineered herpes simplex viruses to the treatment of experimental brain tumors," PNAS USA 93:11313-11318, October 1996, Robertson et al. "Epstein-Barr virus vectors for gene delivery to B lymphocytes," PNAS USA 93:11334-11340, October 1996, Frolov et al., "Alphavirus-based expression vectors: Strategies and applications," PNAS USA 93:11371-11377, October 1996, Kitson et al., J. Virol. 65, 3068-3075, 1991; U.S. Patent Nos. 5,591,439, 5,552,143, Grunhaus et al., 1992, "Adenovirus as cloning vectors," Seminars in Virology (Vol. 3) p. 237-52, 1993, Ballay et al. EMBO Journal, vol. 4, p. 3861-65, Graham, Tibtech 8, 85-87, April, 1990, Prevec et al., J. Gen Virol. 70, 429-434, PCT WO91/11525, Felgner et al. (1994), J. Biol. Chem. 269, 2550-2561, Science, 259:1745-49, 1993 and McClements et

al., "Immunization with DNA vaccines encoding glycoprotein D or glycoprotein B, alone or in combination, induces protective immunity in animal models of herpes simplex virus-2 disease," PNAS USA 93:11414-11420, October 1996, and U.S. Patents Nos 5,591,639, 5,589,466, and 5,580,859 relating to DNA expression vectors, *inter alia*. See also U.S. applications Serial Nos. 08/675,566 and 08/675,556, relating to vectors, including adenovirus vectors.

As to the inserted nucleic acid molecule in a vector of the invention, e.g., the foreign gene, the heterologous or exogenous nucleic acid molecule, e.g., DNA, in vectors of the instant invention, preferably encodes an expression product comprising: an epitope of interest, a biological response modulator, a growth factor, a recognition sequence, a therapeutic gene or a fusion protein. With respect to these terms, reference is made to the following discussion, and generally to Kendrew, THE ENCYCLOPEDIA OF MOLECULAR BIOLOGY (Blackwell Science Ltd., 1995) and Sambrook, Fritsch and Maniatis, <u>Molecular Cloning, A Laboratory Manual</u>, 2nd Ed., Cold Spring Harbor Laboratory Press, 1982.

An epitope of interest is an immunologically relevant region of an antigen or immunogen or immunologically active fragment thereof, e.g., from a pathogen or toxin of veterinary or human interest.

An epitope of interest can be prepared from an antigen of a pathogen or toxin, or from another antigen or toxin which elicits a response with respect to the pathogen, or from another antigen or toxin which elicits a response with respect to the pathogen, such as, for instance: a Morbillivirus antigen, e.g., a canine distemper virus or measles or rinderpest antigen such as HA or F; a rabies glycoprotein, e.g., rabies glycoprotein G; an avian influenza antigen, e.g., turkey influenza HA, Chicken/Pennsylvania/1/83 influenza antigen such as a nudeoprotein (NP); a bovine leukemia virus antigen, e.g.,

gp51,30 envelope; a Newcastle Disease Virus (NDV) antigen, e.g., HN or F; a feline leukemia virus antigen (FeLV), e.g., FeLV envelope protein; RAV-1 env; matrix and/or preplomer of infectious bronchitis virus; a Herpesvirus glycoprotein, e.g., a glycoprotein from feline herpesvirus, equine herpesvirus, bovine herpesvirus, pseudorabies virus, canine herpesvirus, HSV, Marek's Disease Virus, or cytomegalovirus; a flavivirus antigen, e.g., a Japanese encephalitis virus (JEV) antigen, a Yellow Fever antigen, or a Dengue virus antigen; a malaria (*Plasmodium*) antigen, an immunodeficiency virus antigen, e.g., a feline immunodeficiency virus (FIV) antigen or a simian immunodeficiency virus (SIV) antigen or a human immunodeficiency virus antigen (HIV); a parvovirus antigen, e.g., canine parvovirus; an equine influenza antigen; an poxvirus antigen, e.g., an ectromelia antigen, a canarypox virus antigen or a fowlpox virus antigen; or an infectious bursal disease virus antigen, e.g., VP2, VP3, VP4.

An epitope of interest can be from an antigen of a human pathogen or toxin, or from another antigen or toxin which elicits a response with respect to the pathogen, or from another antigen or toxin which elicits a response with respect to the pathogen, such as, for instance: a Morbillivirus antigen, e.g., a measles virus antigen such as HA or F; a rabies glycoprotein, e.g., rabies virus glycoprotein G; an influenza antigen, e.g., influenza virus HA or N; a Herpesvirus antigen, e.g., a glycoprotein of a herpes simplex virus (HSV), a human cytomegalovirus (HCMV), Epstein-Barr; a flavivirus antigen, a JEV, Yellow Fever virus or Dengue virus antigen; a Hepatitis virus antigen, e.g., HBsAg; an immunodeficiency virus antigen, e.g., an HIV antigen such as gp120, gp160; a Hantaan virus antigen; a *C. tetani* antigen; a mumps antigen; a pneumococcal antigen, e.g., PspA; a *Borrelia* antigen, e.g., OspA, OspB, OspC of

Borrelia associated with Lyme disease such as *Borrelia burgdorferi*, *Borrelia afzelli* and *Borrelia garinii*; a chicken pox (varicella zoster) antigen; or a *Plasmodium* antigen.

Of course, the foregoing lists are intended as exemplary, as the epitope of interest can be derived from any antigen of any veterinary or human pathogen; and, to obtain an epitope of interest, one can express an antigen of any veterinary or human pathogen (such that the invention encompasses the exogenous or foreign nucleic acid molecule(s) of interest encoding at least one antigen).

Since the heterologous DNA can be a growth factor or therapeutic gene, the inventive recombinants can be used in gene therapy. Gene therapy involves transferring genetic information; and, with respect to gene therapy and immunotherapy, reference is made to U.S. Patent No. 5,252,479, which is incorporated herein by reference, together with the documents cited in it and on its face, and to WO 94/16716 and allowed U.S. application Serial No. 08/184,009, filed January 19, 1994, each of which is also incorporated herein by reference, together with the documents cited therein. The growth factor or therapeutic gene, for example, can encode a disease-fighting protein, a molecule for treating cancer, a tumor suppressor, a cytokine, a tumor associated antigen, or interferon; and, the growth factor or therapeutic gene can, for example, be selected from the group consisting of a gene encoding alpha-globin, beta-globin, gamma-globin, granulocyte macrophage-colony stimulating factor, tumor necrosis factor, an interleukin, macrophage colony stimulating factor, granulocyte colony stimulating factor, erythropoietin, mast cell growth factor, tumor suppressor p53, retinoblastoma, interferon, melanoma associated antigen or B7.

The invention further relates to an immunogenic, immunological or vaccine composition

containing the inventive vector and an acceptable carrier or diluent(e.g., veterinary acceptable or pharmaceutically acceptable). An immunological composition containing the vector (or an expression product thereof) elicits an immunological response - local or systemic. The response can, but need not be protective. An immunogenic composition containing the inventive recombinants (or an expression product thereof) likewise elicits a local or systemic immunological response which can, but need not be, protective. A vaccine composition elicits a local or systemic protective response. Accordingly, the terms "immunological composition" and "immunogenic composition" include a "vaccine composition" (as the two former terms can be protective compositions).

The invention therefore also provides a method for inducing an immunological response in a host vertebrate comprising administering to the host an immunogenic, immunological or vaccine composition comprising the inventive recombinant virus or vector and an acceptable carrier or diluent. For purposes of this specification, "animal" includes all vertebrate species, except humans; and "vertebrate" includes all vertebrates, including animals (as "animal" is used herein) and humans. And, of course, a subset of "animal" is "mammal", which for purposes of this specification includes all mammals, except humans.

For human administration, the inventive recombinants or vectors, can provide the advantage of expression without productive replication. This thus provides the ability to use recombinants of the invention in immunocompromised individuals; and, provides a level of safety to workers in contact with recombinants of the invention. Therefore, the invention comprehends methods for amplifying or expressing a protein by administering or inoculating a host with a recombinant virus or vector, whereby the host is not a natural host of the recombinant

virus or vector, and there is expression without productive replication.

The exogenous or heterologous DNA (or DNA foreign to vaccine virus) can be DNA encoding any of the aforementioned epitopes of interest, as listed above. In this regard, with respect to *Borrelia* DNA, reference is made to U.S. Patent No. 5,523,089, WO93/08306, PCT/US92/08697, Molecular Microbiology (1989), 3(4), 479-486, and PCT publications WO 93/04175, and WO 96/06165, incorporated herein by reference.

With respect to pneumococcal epitopes of interest, reference is made to Briles et al. WO 92/14488, incorporated herein by reference, with respect to tumor viruses reference is made to Molecular Biology of Tumor Viruses, RNA TUMOR VIRUSES (Second Edition, Edited by Weiss et al., Cold Spring Harbor Laboratory 1982) (e.g., page 44 *et seq.* - Taxonomy of Retroviruses), incorporated herein by reference.

With respect to DNA encoding epitopes of interest, attention is directed to documents cited herein, see, e.g., documents cited *supra* and documents cited *infra*, for instance: U.S. Patents Nos. 5,174,993 and 5,505,941 (e.g., recombinant avipox virus, vaccinia virus; rabies glycoprotein (G), gene, turkey influenza hemagglutinin gene, gp51,30 envelope gene of bovine leukemia virus, Newcastle Disease Virus (NDV) antigen, FelV envelope gene, RAV-1 env gene, NP (nudeoprotein gene of Chicken/Pennsylvania/1/83 influenza virus), matrix and preplomer gene of infectious bronchitis virus; HSV gD), U.S. Patent No. 5,338,683 (e.g., recombinant vaccinia virus, avipox virus; DNA encoding Herpesvirus glycoproteins, *inter alia*), U.S. Patent No. 5,494,807 (e.g., recombinant vaccinia, avipox; exogenous DNA encoding antigens from rabies, Hepatitis B, JEV, YF, Dengue, measles, pseudorabies, Epstein-Barr, HSV, HIV, SIV, EHV, BHV, HCMV, canine parvovirus, equine influenza, FeLV, FHV, Hantaan, *C. tetani*, avian influenza, mumps,

NDV, *inter alia*), U.S. Patent No. 5,503,834 (e.g., recombinant vaccinia, avipox, Morbillivirus, e.g., measles F, hemagglutinin, *inter alia*), U.S. Patent No. 4,722,848 (e.g., recombinant vaccinia virus; HSV tk, HSV glycoproteins, e.g., gB, gD, influenza HA, Hepatitis B, e.g., HBsAg, *inter alia*), U.K. Patent GB 2 269 820 B and U.S. Patent No. 5,514,375 (recombinant poxvirus; flavivirus structural proteins); WO 92/22641 and U.S. applications Serial Nos. 08/417,210 and 08/372,664 (e.g., recombinant poxvirus; immunodeficiency virus, HTLV, *inter alia*), WO 93/03145 and allowed U.S. applications 08/204,729 and 08/303,124 (e.g., recombinant poxvirus; IBDV, *inter alia*), WO 94/16716 and allowed U.S. application Serial No. 08/184,009, filed January 19, 1994 (e.g., recombinant poxvirus; cytokine and/or tumor associated antigens, *inter alia*), U.S. application Serial No. 08/469,969 (rabies combination compositions), U.S. application Serial No. 08/746,668 (lentivirus, retrovirus and/or immunodeficiency virus, including feline immunodeficiency virus, *inter alia*), U.S. Patent No. 5,529,780 and allowed U.S. application Serial No. 08/413,118 (canine herpesvirus), U.S. application Serial No. 08/471,025 (calicivirus), WO 96/3941 and U.S. application Serial No. 08/658,665 (cytomegalovirus), and PCT/US94/06652 (*Plasmodium* antigens such as from each stage of the *Plasmodium* life cycle).

As to antigens for use in vaccine or immunological compositions, reference is made to the documents and discussion set forth in the documents cited herein (see, e.g., documents cited *supra*); see also Stedman's Medical Dictionary (24th edition, 1982, e.g., definition of vaccine (for a list of antigens used in vaccine formulations; such antigens or epitopes of interest from those antigens can be used in the invention, as either an expression product of an inventive recombinant virus or vector, or in a multivalent composition containing an inventive

recombinant virus or vector or an expression product therefrom).

As to epitopes of interest, one skilled in the art can determine an epitope or immunodominant region of a peptide or polypeptide and ergo the coding DNA therefor from the knowledge of the amino acid and corresponding DNA sequences of the peptide or polypeptide, as well as from the nature of particular amino acids (e.g., size, charge, etc.) and the codon dictionary, without undue experimentation.

A general method for determining which portions of a protein to use in an immunological composition focuses on the size and sequence of the antigen of interest. "In general, large proteins, because they have more potential determinants are better antigens than small ones. The more foreign an antigen, that is the less similar to self configurations which induce tolerance, the more effective it is in provoking an immune response." Ivan Roitt, Essential Immunology, 1988.

As to size: the skilled artisan can maximize the size of the protein encoded by the DNA sequence to be inserted into the viral vector (keeping in mind the packaging limitations of the vector). To minimize the DNA inserted while maximizing the size of the protein expressed, the DNA sequence can exclude introns (regions of a gene which are transcribed but which are subsequently excised from the primary RNA transcript).

At a minimum, the DNA sequence can code for a peptide at least 8 or 9 amino acids long. This is the minimum length that a peptide needs to be in order to stimulate a CD8+ T cell response (which recognizes virus infected cells or cancerous cells). A minimum peptide length of 13 to 25 amino acids is useful to stimulate a CD4+ T cell response (which recognizes special antigen presenting cells which have engulfed the pathogen). See Kendrew, *supra*. However, as these are minimum lengths, these peptides are likely to generate an immunological

response, i.e., an antibody or T cell response; but, for a protective response (as from a vaccine composition), a longer peptide is preferred.

With respect to the sequence, the DNA sequence preferably encodes at least regions of the peptide that generate an antibody response or a T cell response. One method to determine T and B cell epitopes involves epitope mapping. The protein of interest "is fragmented into overlapping peptides with proteolytic enzymes. The individual peptides are then tested for their ability to bind to an antibody elicited by the native protein or to induce T cell or B cell activation. This approach has been particularly useful in mapping T-cell epitopes since the T cell recognizes short linear peptides complexed with MHC molecules. The method is less effective for determining B-cell epitopes" since B cell epitopes are often not linear amino acid sequence but rather result from the tertiary structure of the folded three dimensional protein. Janis Kuby, Immunology, (1992) pp. 79-80.

Another method for determining an epitope of interest is to choose the regions of the protein that are hydrophilic. Hydrophilic residues are often on the surface of the protein and are therefore often the regions of the protein which are accessible to the antibody. Janis Kuby, Immunology, (1992) p. 81

Yet another method for determining an epitope of interest is to perform an X-ray crystallographic analysis of the antigen (full length)-antibody complex. Janis Kuby, Immunology, (1992) p. 80.

Still another method for choosing an epitope of interest which can generate a T cell response is to identify from the protein sequence potential HLA anchor binding motifs which are peptide sequences which are known to be likely to bind to the MHC molecule.

The peptide which is a putative epitope of interest, to generate a T cell response, should be

presented in a MHC complex. The peptide preferably contains appropriate anchor motifs for binding to the MHC molecules, and should bind with high enough affinity to generate an immune response. Factors which can be considered are: the HLA type of the patient (vertebrate, animal or human) expected to be immunized, the sequence of the protein, the presence of appropriate anchor motifs and the occurrence of the peptide sequence in other vital cells.

An immune response is generated, in general, as follows: T cells recognize proteins only when the protein has been cleaved into smaller peptides and is presented in a complex called the "major histocompatability complex MHC" located on another cell's surface. There are two classes of MHC complexes - class I and class II, and each class is made up of many different alleles. Different patients have different types of MHC complex alleles; they are said to have a 'different HLA type.'

Class I MHC complexes are found on virtually every cell and present peptides from proteins produced inside the cell. Thus, Class I MHC complexes are useful for killing cells which when infected by viruses or which have become cancerous and as the result of expression of an oncogene. T cells which have a protein called CD8 on their surface, bind specifically to the MHC class I/peptide complexes via the T cell receptor. This leads to cytolytic effector activities.

Class II MHC complexes are found only on antigen- presenting cells and are used to present peptides from circulating pathogens which have been endocytosed by the antigen- presenting cells. T cells which have a protein called CD4 bind to the MHC class II/peptide complexes via the T cell receptor. This leads to the synthesis of specific cytokines which stimulate an immune response.

Some guidelines in determining whether a protein is an epitopes of interest which will stimulate a

T cell response, include: Peptide length - the peptide should be at least 8 or 9 amino acids long to fit into the MHC class I complex and at least 13-25 amino acids long to fit into a class II MHC complex. This length is a minimum for the peptide to bind to the MHC complex. It is preferred for the peptides to be longer than these lengths because cells may cut the expressed peptides. The peptide should contain an appropriate anchor motif which will enable it to bind to the various class I or class II molecules with high enough specificity to generate an immune response (See Bocchia, M. et al, <u>Specific Binding of Leukemia Oncogene Fusion Protein Peptides to HLA Class I Molecules</u>, Blood 85:2680-2684; Englehard, VH, <u>Structure of peptides associated with class I and class II MHC molecules</u> Ann. Rev. Immunol. 12:181 (1994)). This can be done, without undue experimentation, by comparing the sequence of the protein of interest with published structures of peptides associated with the MHC molecules. Protein epitopes recognized by T cell receptors are peptides generated by enzymatic degradation of the protein molecule and are presented on the cell surface in association with class I or class II MHC molecules.

Further, the skilled artisan can ascertain an epitope of interest by comparing the protein sequence with sequences listed in the protein data base.

Even further, another method is simply to generate or express portions of a protein of interest, generate monoclonal antibodies to those portions of the protein of interest, and then ascertain whether those antibodies inhibit growth *in vitro* of the pathogen from which the from which the protein was derived. The skilled artisan can use the other guidelines set forth in this disclosure and in the art for generating or expressing portions of a protein of interest for analysis as to whether antibodies thereto inhibit growth *in vitro*. For example, the skilled artisan can generate portions of

a protein of interest by: selecting 8 to 9 or 13 to 25 amino acid length portions of the protein, selecting hydrophylic regions, selecting portions shown to bind from X-ray data of the antigen (full length)-antibody complex, selecting regions which differ in sequence from other proteins, selecting potential HLA anchor binding motifs, or any combination of these methods or other methods known in the art.

Epitopes recognized by antibodies are expressed on the surface of a protein. To determine the regions of a protein most likely to stimulate an antibody response one skilled in the art can preferably perform an epitope map, using the general methods described above, or other mapping methods known in the art.

As can be seen from the foregoing, without undue experimentation, from this disclosure and the knowledge in the art, the skilled artisan can ascertain the amino acid and corresponding DNA sequence of an epitope of interest for obtaining a T cell, B cell and/or antibody response. In addition, reference is made to Gefter et al., U.S. Patent No. 5,019,384, issued May 28, 1991, and the documents it cites, incorporated herein by reference (Note especially the "Relevant Literature" section of this patent, and column 13 of this patent which discloses that: "A large number of epitopes have been defined for a wide variety of organisms of interest. Of particular interest are those epitopes to which neutralizing antibodies are directed. Disclosures of such epitopes are in many of the references cited in the Relevant Literature section.")

With respect to expression of a biological response modulator, reference is made to Wohlstadter, "Selection Methods," WO 93/19170, published 30 September 1993, and the documents cited therein, incorporated herein by reference.

For instance, a biological response modulator modulates biological activity; for instance, a biological

response modulator is a modulatory component such as a high molecular weight protein associated with non-NMDA excitatory amino acid receptors and which allosterically regulates affinity of AMPA binding (See Kendrew, *supra*). The recombinant of the present invention can express such a high molecular weight protein.

More generally, nature has provided a number of precedents of biological response modulators. Modulation of activity may be carried out through mechanisms as complicated and intricate as allosteric induced quaternary change to simple presence/absence, e.g., expression/degradation, systems. Indeed, the repression/activation of expression of many biological molecules is itself mediated by molecules whose activities are capable of being modulated through a variety of mechanisms.

Table 2 of Neidhardt et al *Physiology of the Bacterial Cell* (Sinauer Associates Inc., Publishers, 1990), at page 73, lists chemical modifications to bacterial proteins. As is noted in that table, some modifications are involved in proper assembly and other modifications are not, but in either case such modifications are capable of causing modulation of function. From that table, analogous chemical modulations for proteins of other cells can be determined, without undue experimentation.

In some instances modulation of biological functions may be mediated simply through the proper/improper localization of a molecule. Molecules may function to provide a growth advantage or disadvantage only if they are targeted to a particular location. For example, a molecule may be typically not taken up or used by a cell, as a function of that molecule being first degraded by the cell by secretion of an enzyme for that degradation. Thus, production of the enzyme by a recombinant can regulate use or uptake of the molecule by a cell. Likewise, the recombinant can

express a molecule which binds to the enzyme necessary for uptake or use of a molecule, thereby similarly regulating its uptake or use.

Localization targeting of proteins carried out through cleavage of signal peptides another type of modulation or regulation. In this case, a specific endoprotease catalytic activity can be expressed by the recombinant.

Other examples of mechanisms through which modulation of function may occur are RNA virus poly-proteins, allosteric effects, and general covalent and non-covalent steric hindrance. HIV is a well studied example of an RNA virus which expresses non-functional poly-protein constructs. In HIV "the gag, pol, and env poly-proteins are processed to yield, respectively, the viral structural proteins p17, p24, and p15--reverse transcriptase and integrase--and the two envelope proteins gp41 and gp120" (Kohl et al., PNAS USA 85:4686-90 (1988)). The proper cleavage of the poly-proteins is crucial for replication of the virus, and virions carrying inactive mutant HIV protease are non-infectious (*Id.*). This is another example of the fusion of proteins down-modulating their activity. Thus, it is possible to construct recombinant viruses which express molecules which interfere with endoproteases, or which provide endoproteases, for inhibiting or enhancing the natural expression of certain proteins (by interfering with or enhancing cleavage).

The functional usefulness of enzymes may also be modulated by altering their capability of catalyzing a reaction. Illustrative examples of modulated molecules are zymogens, formation/disassociation of multi-subunit functional complexes, RNA virus poly-protein chains, allosteric interactions, general steric hindrance (covalent and non-covalent) and a variety of chemical modifications such as phosphorylation, methylation, acetylation, adenylation, and uridenylation (see Table 1

of Neidhardt, *supra*, at page 315 and Table 2 at page 73).

Zymogens are examples of naturally occurring protein fusions which cause modulation of enzymatic activity. Zymogens are one class of proteins which are converted into their active state through limited proteolysis. See Table 3 of Reich, *Proteases and Biological Control*, **Vol. 2,** (1975) at page 54). Nature has developed a mechanism of down-modulating the activity of certain enzymes, such as trypsin, by expressing these enzymes with additional "leader" peptide sequences at their amino termini. With the extra peptide sequence the enzyme is in the inactive zymogen state. Upon cleavage of this sequence the zymogen is converted to its enzymatically active state. The overall reaction rates of the zymogen are "about 10^5-10^6 times lower than those of the corresponding enzyme" (See Table 3 of Reich, *supra* at page 54).

It is therefore possible to down-modulate the function of certain enzymes simply by the addition of a peptide sequence to one of its termini. For example, with knowledge of this property, a recombinant can express peptide sequences containing additional amino acids at one or both termini.

The formation or disassociation of multi-subunit enzymes is another way through which modulation may occur. Different mechanisms may be responsible for the modulation of activity upon formation or disassociation of multi-subunit enzymes.

Therefore, sterically hindering the proper specific subunit interactions will down-modulate the catalytic activity. And accordingly, the recombinant of the invention can express a molecule which sterically hinders a naturally occurring enzyme or enzyme complex, so as to modulate biological functions.

Certain enzyme inhibitors afford good examples of functional down-modulation through covalent steric hindrance or modification. Suicide substrates which

irreversibly bind to the active site of an enzyme at a catalytically important amino acid in the active site are examples of covalent modifications which sterically block the enzymatic active site. An example of a suicide substrate is TPCK for chymotrypsin (Fritsch, *Enzyme Structure and Mechanism*, 2d ed; Freeman & Co. Publishers, 1984)). This type of modulation is possible by the recombinant expressing a suitable suicide substrate, to thereby modulate biological responses (e.g., by limiting enzyme activity).

There are also examples of non-covalent steric hindrance including many repressor molecules. The recombinant can express repressor molecules which are capable of sterically hindering and thus down-modulating the function of a DNA sequence by preventing particular DNA-RNA polymerase interactions.

Allosteric effects are another way through which modulation is carried out in some biological systems. Aspartate transcarbamoylase is a well characterized allosteric enzyme. Interacting with the catalytic subunits are regulatory domains. Upon binding to CTP or UTP the regulatory subunits are capable of inducing a quaternary structural change in the holoenzyme causing down-modulation of catalytic activity. In contrast, binding of ATP to the regulatory subunits is capable of causing up-modulation of catalytic activity (Fritsch, *supra*). Using methods of the invention, molecules can be expressed which are capable of binding and causing modulatory quaternary or tertiary changes.

In addition, a variety of chemical modifications, e.g., phosphorylation, methylation, acetylation, adenylation, and uridenylation may be carried out so as to modulate function. It is known that modifications such as these play important roles in the regulation of many important cellular components. Table 2 of Neidhardt, *supra*, at page 73, lists different bacterial enzymes which undergo such modifications. From

that list, one skilled in the art can ascertain other enzymes of other systems which undergo the same or similar modifications, without undue experimentation. In addition, many proteins which are implicated in human disease also undergo such chemical modifications. For example, many oncogenes have been found to be modified by phosphorylation or to modify other proteins through phosphorylation or dephosphorylation. Therefore, the ability afforded by the invention to express modulators which can modify or alter function, e.g., phosphorylation, is of importance.

From the foregoing, the skilled artisan can use the present invention to express a biological response modulator, without any undue experimentation.

With respect to expression of fusion proteins by inventive recombinants, reference is made to Sambrook, Fritsch, Maniatis, *Molecular Cloning, A LABORATORY MANUAL* (2d Edition, Cold Spring Harbor Laboratory Press, 1989) (especially Volume 3), and Kendrew, *supra*, incorporated herein by reference. The teachings of Sambrook et al., can be suitably modified, without undue experimentation, from this disclosure, for the skilled artisan to generate recombinants or vectors expressing fusion proteins.

With regard to gene therapy and immunotherapy, reference is made to U.S. Patent Nos. 4,690,915 and 5,252,479, which are incorporated herein by reference, together with the documents cited therein it and on their face, and to WO 94/16716 and U.S. application Serial No. 08/184,009, filed January 19, 1994, each of which is also incorporated herein by reference, together with the documents cited therein.

A growth factor can be defined as multifunctional, locally acting intercellular signalling peptides which control both ontogeny and maintenance of tissue and function (see Kendrew, *supra*, especially at page 455 *et seq.*).

The growth factor or therapeutic gene, for

example, can encode a disease-fighting protein, a molecule for treating cancer, a tumor suppressor, a cytokine, a tumor associated antigen, or interferon; and, the growth factor or therapeutic gene can, for example, be selected from the group consisting of a gene encoding alpha-globin, beta-globin, gamma-globin, granulocyte macrophage-colony stimulating factor, tumor necrosis factor, an interleukin (e.g., an interleukin selected from interleukins 1 to 14, or 1 to 11, or any combination thereof), macrophage colony stimulating factor, granulocyte colony stimulating factor, erythropoietin, mast cell growth factor, tumor suppressor p53, retinoblastoma, interferon, melanoma associated antigen or B7. U.S. Patent No. 5,252,479 provides a list of proteins which can be expressed in an adenovirus system for gene therapy, and the skilled artisan is directed to that disclosure. WO 94/16716 and allowed U.S. application Serial No. 08/184,009, filed January 19, 1994, provide genes for cytokines and tumor associated antigens and immunotherapy methods, including *ex vivo* methods, and the skilled artisan is directed to those disclosures.

Thus, one skilled in the art can create recombinants or vectors expressing a growth factor or therapeutic gene and use the recombinants or vectors, from this disclosure and the knowledge in the art, without undue experimentation.

Moreover, from the foregoing and the knowledge in the art, no undue experimentation is required for the skilled artisan to construct an inventive recombinant or vector which expresses an epitope of interest, a biological response modulator, a growth factor, a recognition sequence, a therapeutic gene, or a fusion protein; or for the skilled artisan to use such a recombinant or vector.

As the recombinants or vectors of the invention can be used for expression of gene products *in vitro*,

techniques for protein purification can be employed in the practice of the invention, and such techniques, in general, include:

Briefly, the cells are disrupted and the protein of interest is released into an aqueous "extract". There are many methods of cellular disintegration, which vary from relatively gentle to vigorous conditions, and the choice of one method over the other is dependent upon the source material. Animal tissues vary from the very easily broken erythrocytes to tough collagenous material such as found in blood vessels and other smooth-muscle containing tissue. Bacteria vary from fairly fragile organisms that can be broken up by digestive enzymes or osmotic shock to more resilient species with thick cell walls, needing vigorous mechanical treatment for disintegration.

Gentle techniques include cell lysis, enzymatic digestion, chemical solubilization, hand homogenization and mincing (or grinding); moderate techniques of cell disintegration include blade homogenization and grinding with abrasive materials, i.e., sand or alumina; and vigorous techniques include french press, ultrasonication, bead mill or Manton-Gaulin homogenization. Each of the aforementioned techniques are art-recognized, and it is well within the scope of knowledge of the skilled artisan to determine the appropriate method for cell disintegration based upon the starting material, and the teachings herein and in the art.

Following cell disintegration, the extract is prepared by centrifuging off insoluble material. At this stage, one may proceed with the purification method, as an extract containing as much of the protein of interest as possible has been prepared, and, where appropriate, particulate and most nonprotein materials have been removed.

Standard techniques of protein purification may

be employed to further purify the protein of interest, including: precipitation by taking advantage of the solubility of the protein of interest at varying salt concentrations, precipitation with organic solvents, polymers and other materials, affinity precipitation and selective denaturation; column chromatography, including high performance liquid chromatography (HPLC), ion-exchange, affinity, immuno affinity or dye-ligand chromatography; immunoprecipitation and the use of gel filtration, electrophoretic methods, ultrafiltration and isoelectric focusing. Each of the above-identified methods are well within the knowledge of the skilled artisan, and no undue experimentation is required to purify the proteins or epitopes of interest from expression of a recombinant or vector of the invention, using the standard methodologies outlined hereinabove, and in the literature, as well as the teachings in the Examples below.

As the expression products can provide an antigenic, immunological, or protective (vaccine) response, the invention further relates to products therefrom; namely, antibodies and uses thereof. More in particular, the expression products can elicit antibodies by administration of those products or of recombinants or vectors expressing the products. The antibodies can be monoclonal antibodies; and, the antibodies or expression products can be used in kits, assays, tests, and the like involving binding, so that the invention relates to these uses too. Additionally, since the recombinants or vectors of the invention can be used to replicate DNA, the invention relates to the inventive recombinants as vectors and methods for replicating DNA by infecting or transfecting cells with the recombinant and harvesting DNA therefrom. The resultant DNA can be used as probes or primers or for amplification.

The administration procedure for the inventive recombinants or vectors or expression products thereof,

compositions of the invention such as immunological, antigenic or vaccine compositions or therapeutic compositions can be via a parenteral route (intradermal, intramuscular or subcutaneous). Such an administration enables a systemic immune response. The administration can be via a mucosal route, e.g., oral, nasal, genital, etc. Such an administration enables a local immune response.

More generally, the inventive antigenic, immunological or vaccine compositions or therapeutic compositions can be prepared in accordance with standard techniques well known to those skilled in the pharmaceutical, medical or veterinary arts. Such compositions can be administered in dosages and by techniques well known to those skilled in the medical or veterinary arts taking into consideration such factors as the breed or species, age, sex, weight, and condition of the particular patient, and the route of administration. The compositions can be administered alone, or can be co-administered or sequentially administered with other compositions of the invention or with other immunological, antigenic or vaccine or therapeutic compositions. Such other compositions can include purified native antigens or epitopes or antigens or epitopes from expression by an inventive recombinant or vector or another vector system; and are administered taking into account the aforementioned factors.

Examples of compositions of the invention include liquid preparations for orifice, e.g., oral, nasal, anal, genital, e.g., vaginal, etc., administration such as suspensions, syrups or elixirs; and, preparations for parenteral, subcutaneous, intradermal, intramuscular or intravenous administration (e.g., injectable administration) such as sterile suspensions or emulsions. In such compositions the recombinant or vector may be in admixture with a suitable carrier, diluent, or excipient such as sterile water, physiological saline, glucose or

the like.

Antigenic, immunological or vaccine compositions typically can contain an adjuvant and an amount of the recombinant or vector or expression product to elicit the desired response. In human applications, alum (aluminum phosphate or aluminum hydroxide) is a typical adjuvant. Saponin and its purified component Quil A, Freund's complete adjuvant and other adjuvants used in research and veterinary applications have toxicities which limit their potential use in human vaccines. Chemically defined preparations such as muramyl dipeptide, monophosphoryl lipid A, phospholipid conjugates such as those described by Goodman-Snitkoff et al. J. Immunol. 147:410-415 (1991) and incorporated by reference herein, encapsulation of the protein within a proteoliposome as described by Miller et al., J. Exp. Med. 176:1739-1744 (1992) and incorporated by reference herein, and encapsulation of the protein in lipid vesicles such as Novasome™ lipid vesicles (Micro Vescular Systems, Inc., Nashua, NH) can also be used.

The composition may be packaged in a single dosage form for immunization by parenteral (i.e., intramuscular, intradermal or subcutaneous) administration or orifice administration, e.g., perlingual (i.e., oral), intragastric, mucosal including intraoral, intraanal, intravaginal, and the like administration. And again, the effective dosage and route of administration are determined by the nature of the composition, by the nature of the expression product, by expression level if the recombinant is directly used, and by known factors, such as breed or species, age, sex, weight, condition and nature of host, as well as LD_{50} and other screening procedures which are known and do not require undue experimentation. Dosages of expressed product can range from a few to a few hundred micrograms, e.g., 5 to 500 μg. The inventive recombinant or vector can be administered in any suitable amount to achieve

expression at these dosage levels. The viral recombinants of the invention can be administered in an amount of about $10^{3.5}$ pfu; thus, the inventive viral recombinant is preferably administered in at least this amount; more preferably about 10^4 pfu to about 10^6 pfu; however higher dosages such as about 10^4 pfu to about 10^{10} pfu, e.g., about 10^5 pfu to about 10^9 pfu, for instance about 10^6 pfu to about 10^8 pfu can be employed. Suitable quantities of inventive plasmid or naked DNA in plasmid or naked DNA compositions can be 1 ug to 100 mg; preferably 0.1 to 10 mg, but lower levels such as 0.1 to 2 mg or preferably 1-10 ug may be employed Other suitable carriers or diluents can be water or a buffered saline, with or without a preservative. The expression product or recombinant or vector may be lyophilized for resuspension at the time of administration or can be in solution.

The carrier may also be a polymeric delayed release system. Synthetic polymers are particularly useful in the formulation of a composition having controlled release. An early example of this was the polymerization of methyl methacrylate into spheres having diameters less than one micron to form so-called nano particles, reported by Kreuter, J., Microcapsules and Nanoparticles in Medicine and Pharmacology, M. Donbrow (Ed). CRC Press, p. 125-148.

Microencapsulation has been applied to the injection of microencapsulated pharmaceuticals to give a controlled release. A number of factors contribute to the selection of a particular polymer for microencapsulation. The reproducibility of polymer synthesis and the microencapsulation process, the cost of the microencapsulation materials and process, the toxicological profile, the requirements for variable release kinetics and the physicochemical compatibility of the polymer and the antigens are all factors that must be considered. Examples of useful polymers are

polycarbonates, polyesters, polyurethanes, polyorthoesters and polyamides, particularly those that are biodegradable.

A frequent choice of a carrier for pharmaceuticals and more recently for antigens is poly (d,1-lactide-co-glycolide) (PLGA). This is a biodegradable polyester that has a long history of medical use in erodible sutures, bone plates and other temporary prostheses where it has not exhibited any toxicity. A wide variety of pharmaceuticals including peptides and antigens have been formulated into PLGA microcapsules. A body of data has accumulated on the adaption of PLGA for the controlled release of antigen, for example, as reviewed by Eldridge, J.H., et al. Current Topics in Microbiology and Immunology, 1989, 146:59-66. The entrapment of antigens in PLGA microspheres of 1 to 10 microns in diameter has been shown to have a remarkable adjuvant effect when administered orally. The PLGA microencapsulation process uses a phase separation of a water-in-oil emulsion. The compound of interest is prepared as an aqueous solution and the PLGA is dissolved in a suitable organic solvents such as methylene chloride and ethyl acetate. These two immiscible solutions are co-emulsified by high-speed stirring. A non-solvent for the polymer is then added, causing precipitation of the polymer around the aqueous droplets to form embryonic microcapsules. The microcapsules are collected, and stabilized with one of an assortment of agents (polyvinyl alcohol (PVA), gelatin, alginates, polyvinylpyrrolidone (PVP), methyl cellulose) and the solvent removed by either drying *in vacuo* or solvent extraction.

Thus, solid, including solid-containing-liquid, liquid, and gel (including "gel caps") compositions are envisioned.

Furthermore, the inventive vectors or recombinants can be used in any desired immunization or

administration regimen; e.g., as part of periodic vaccinations such as annual vaccinations as in the veterinary arts or as in periodic vaccinations as in the human medical arts, or as in a prime-boost regimen wherein an inventive vector or recombinant is administered either before or after the administration of the same or of a different epitope of interest or recombinant or vector expressing such a same or different epitope of interest (including an inventive recombinant or vector expressing such a same or different epitope of interest), see, e.g., documents cited herein such as U.S. application Serial No. 08/746,668.

Additionally, the inventive vectors or recombinants and the expression products therefrom can stimulate an immune or antibody response in animals. From those antibodies, by techniques well-known in the art, monoclonal antibodies can be prepared and, those monoclonal antibodies, can be employed in well known antibody binding assays, diagnostic kits or tests to determine the presence or absence of antigen(s) and therefrom the presence or absence of the natural causative agent of the antigen or, to determine whether an immune response to that agent or to the antigen(s) has simply been stimulated.

Monoclonal antibodies are immunoglobulin produced by hybridoma cells. A monoclonal antibody reacts with a single antigenic determinant and provides greater specificity than a conventional, serum-derived antibody. Furthermore, screening a large number of monoclonal antibodies makes it possible to select an individual antibody with desired specificity, avidity and isotype. Hybridoma cell lines provide a constant, inexpensive source of chemically identical antibodies and preparations of such antibodies can be easily standardized. Methods for producing monoclonal antibodies are well known to those of ordinary skill in the art, e.g., Koprowski, H. et al., U.S. Pat. No.

4,196,265, issued Apr. 1, 1989, incorporated herein by reference.

Uses of monoclonal antibodies are known. One such use is in diagnostic methods, e.g., David, G. and Greene, H., U.S. Pat. No. 4,376,110, issued Mar. 8, 1983, incorporated herein by reference.

Monoclonal antibodies have also been used to recover materials by immunoadsorption chromatography, e.g. Milstein, C., 1980, Scientific American 243:66, 70, incorporated herein by reference.

Furthermore, the inventive recombinants or vectors or expression products therefrom can be used to stimulate a response in cells *in vitro* or *ex vivo* for subsequent reinfusion into a patient. If the patient is seronegative, the reinfusion is to stimulate an immune response, e.g., an immunological or antigenic response such as active immunization. In a seropositive individual, the reinfusion is to stimulate or boost the immune system against a pathogen.

The recombinants or vectors of the invention are also useful for generating DNA for probes or for PCR primers which can be used to detect the presence or absence of hybridizable DNA or to amplify DNA, e.g., to detect a pathogen in a sample or for amplifying DNA.

Since viruses require translation of viral mRNAs in order to generate viral proteins required for replication, it is evident that any function which blocks the action of PKR in the infected cell will have a positive effect on viral protein expression. Thus, co-expression, in some fashion, of the vaccinia E3L/K3L gene products, or a homolog of E3L and/or K3L, may provide a general mechanism for enhancing the expression levels of heterologous gene products by vectors in general. The E3L/K3L or homologous functions may enhance or augment native anti-PKR mechanisms, and thus increase protein expression levels and/or persistence. This provides a useful element towards optimizing the efficiency of

eukaryotic virus systems as immunization vehicles. This approach could be further extended for improvement of DNA-based immunogens, e.g., naked DNA or plasmid DNA vector systems. Further, employing a nucleotide sequence for a transcription factor, e.g., for an early and/or late viral transcription factor, in conjunction with enhancing translation by employing a nucleotide sequence for a translation factor, can even further enhance or increase expression by increasing or enhancing transcription and translation; and thus, increasing or enhancing levels or persistence of expression can be obtained.

A better understanding of the present invention and of its many advantages will be had from the following non-limiting Examples, given by way of illustration.

EXAMPLES

Example 1 - NYVAC Recombinants Containing H4L

The plasmids placZH6H4L and placZH6H4Lreverse (ATCC Deposit No.______) were used as donor plasmids for *in vivo* recombination with the rescue virus vP994 (ATCC Deposit No. ______; U.S. Patent No. 5,494,807, incorporated herein by reference; vaccinia H6 promoter/HIV1 MN *env*-noncleavable, secreted gp140, in HA insertion site). The donor plasmids were designed to replace the endogenous promoter and coding sequences of H4L by homologous recombination. The resulting recombinant viruses were designated vP1379 and vP1380; vP1379 contains the H6lacZ/H6H4L cassette in a head-to-head configuration; vP1380 contains the H6lacZ/H6H4L cassette in a head-to-tail configuration (SEQ ID NO: ; Fig. 1).

The plasmids were constructed as follows:

H4L Expression Cassette

The H4L open reading frame (orf) as delineated in Goebel et al. 1990 corresponds to positions 94830-92446 in the Copenhagen (vaccine) strain vaccinia virus genomic sequence. pSD404VC contains a clone of the 8.6Kb

*Hind*III H fragment of Copenhagen vaccinia virus inserted into the pUC vector background. pSD404VC was digested with *Pvu*II to isolate a 3860bp fragment containing the H4L coding sequences and flanking sequences. The 3860bp fragment was inserted into the blunted *Bam*HI site of pBSecogpt (*E.coli* gpt gene (ATCC No. 37145) under the control of Copenhagen B13R promoter in the pBS SK vector (Stratagene La Jolla, CA.)) resulting in plasmid pRW935.

pRW935 was linearized with *Eco*RI and partially digested with *Dra*I to remove a 970bp fragment containing the 5′ end of the H4L coding sequence. Using a series of Polymerase Chain Reactions (PCRs) the H4L coding sequence was reengineered to be under the control of the modified vaccinia H6 promoter (Perkus et al. 1989). Using the plasmid template pRW935 and primer pairs RW500/RW502 and RW501/RW503 in the PCR amplifications, the 5′ H4L sequences were regenerated. In addition, the oligonucleotide, RW502, modifies the H4L coding sequences (position 341-348 from the A of the ATG) from TTTTTTTT to TTTTCTTC without altering the predicted amino acid sequence to remove an early transcriptional stop signal (Yuan, L. and Moss, B., 1987). The modified H6 promoter was amplified from the plasmid template pRW936 using oligonucleotides RW504 and RW507. Oligonucleotides RW505 and RW506 having complementary sequences were PCR amplified directly. The four PCR reactions were pooled and further amplified using primer pair RW500 and RW505. The resulting PCR fragment was digested with *Dra*I and *Eco*RI and cloned into *Dra*I and *Eco*RI digested pRW935 generating pRW939. A PCR introduced error in the 5′ end of the coding region of pRW939 was corrected, resulting in plasmid pRW947. Specifically, the PCR error introduced in pRW939 (H4L codon 155 is AAA - correct codon should be GAA) was corrected by replacement of the 600 bp pRW939 *Afl*III-*Eco*RI fragment with the equivalent fragment from pRW935 to generate pRW939. The oligonucleotide sequences for each of the above-

identified oligonucleotides (RW500 and RW501 to RW507; SEQ ID NOS:) are:

RW500 5'- GAAATAGTTAGCGTCAAC -3'
RW501 5'- TGTCTAATGTGTTGAAGAAAAGATCATACAAGTTATAC -3'
RW502 5'- AACTTGTATGATCTTTTCTTCAACACATTAGACATGTATTTAC -3'
RW503 5'- TAAGTTTGTATCGTAATGGACTCTAAAGAGACTATTC -3'
RW504 5'- AGTCTCTTTAGAGTCCATTACGATACAAACTTAAC -3'
RW505
5'-
CCGACGATTTTAAAACGCCACCGTCAGGGAAAGTTTCATAAGAAGCACCGGAAGAGA
AGAGA ATTCTCGGGACAATTGGATC -3'
RW506
5'-
GTCTAGCTGGTGCTGAGTTTCTACGTGAGTTGATTCGTCTCTTGCGTGCCTCTCGTG
ATCCAATTGTCCCGAGATATTCTC -3'
RW507
5'-
GTAGAAACTCAGCACCAGCTAGACAAGCTTCTTTATTCTATACTTAAAAAGTGAAAA
TAAATAC -3'

The plasmid pRW947 was digested with *Xho*I to generate two fragments. The 7036 bp fragment containing the H6 promoted H4L in the pBSSK vector background was purified and self-ligated, resulting in the plasmid pH6H4L. The plasmid pRW973A, containing a LacZ expression cassette under the control of the vaccinia H6 promoter, was digested with *Hind*III. The 3.3 Kbp fragment was purified and ligated into the *Hind*III digested pH6H4L, thereby generating pLACZH6H4Lreverse (H6 promoted LacZ gene and H6 promoted H4L gene in head-to-tail configuration), and placZH6H4L (H6 promoted lacZ gene and H6 promoted H4L gene in a head-to-head configuration).

Example 2 - ALVAC Recombinants

pMPC6H6K3E3 (ATCC No. ____) was used as a donor plasmid in *in vivo* recombination (Piccini et al., 1987) with rescuing virus vCP205 (ATCC No. ___; U.S. application Serial No. 08/417,210, incorporated herein by

reference; HIV expression cassette - vaccinia H6 promoter/HIV truncated *env* MN strain, I3L *gag* with protease in ALVAC C3 insertion site); and the resulting recombinant virus was designated vCP1431A (vaccinia H6/K3L and E3L cassette in the C6 locus).

pC8H6H4 was used as the donor plasmid in *in vivo* recombination with vCP205 and the resulting recombinant virus designated vCP1435 (HIV cassette at C3 locus and the vaccinia H6/H4L expression cassette at C8 locus; H6/H4L expression cassette flanked by ALVAC C8 insertion site sequences (SEQ ID NO:) shown in Fig. 2).

vCP1431A was also used as a rescuing virus in *in vivo* recombination using plasmid pC8H6H4, generating the recombinant designated vCP1437A (HIV cassette at the C3 locus, the H6/K3L and E3L cassette at the C6 locus, and the vaccinia H6/H4L cassette at the C8 locus). With respect to the H6/K3L expression cassette and the vaccinia E3L gene with the endogenous promoter flanked by the ALVAC C6 insertion site sequences reference is made to Fig. 3 (SEQ ID NO:).

pC3H6FHVB (ATCC No. ___; Fig. 5, SEQ ID NO: ; H6 promoted FHV gB ORF with early transcriptional and translational stop signals at both 5′ and 3′ ends flanked by the left and right arms of the ALVAC C3 locus) was used in *in vivo* recombination with the ALVAC (ATCC No. VR-2547) to generate vCP1459 (H6 promoted FHV gB expression cassette in deorfed C3 insertion locus). With respect to the FHV-1 gB coding region in which the two internal T_5NT motifs have been mutated, see Fig. 4 (SEQ ID NO:).

pC3H6FHVB was used in *in vivo* recombination with vCP1431A to generate vCP1460 (H6 promoted FHV gB expression cassette in the deorfed C3 insertion locus and vaccinia E3L/K3L genes in C6 locus).

pC3H6FHVB was used in *in vivo* recombination with vCP1437 to generate vCP1464 (H6 promoted FHV gB

expression cassette in deorfed C3 insertion locus, vaccinia E3L/K3L genes in C6 locus and H6 promoted vaccinia H4L ORF in C8 locus).

pMPC5H6PN (HIV pol/nef "string of beads" cassette in the ALVAC C5 locus) was used in recombination with vCP205 to obtain vCP1433 (ATCC Deposit No. ____). Thus, recombinant ALVAC-MN120TMGNPst (vCP1433) was generated by insertion of an expression cassette encoding a synthetic polypeptide containing all of the known Pol CTL epitopes (Nixon and McMichael; 1991) and all of the known human Nef CTL epitopes into vCP205 at the insertion site known as C5.

pMPC6H6K3E3 (ATCC Deposit No. ___ ; containing vaccinia H6/K3L expression cassette and vaccinia E3L gene with endogenous promoter flanked by the ALVAC C6 insertion site sequences) was used in recombination with vCP1433 to obtain vCP1452. Figures 6 and 7 show the nucleotide and amino acid sequences of the vCP1433 and vCP1452 inserts. Figure 8 shows the K3L E3L in C6 in vCP1452. vCP1452 contains the HIV type 1 *gag* and protease genes derived from the IIIB isolate, the gp120 envelope sequences derived from the MN isolate, and sequences encoding a polypeptide encompassing the known human CTL epitopes from HIV-1 Nef and Pol (Nef1 and Nef2 CTL epitopes, and Pol1, Pol2 and Pol3 CTL epitopes). The expressed gp120 moiety is linked to the transmembrane (TM) anchor sequence (28 amino acids) of the envelope glycoprotein. In addition to the HIV coding sequences vCP1452 contains the vaccinia virus E3L and K3L coding sequences inserted into the C6 site. The insertion sites and promoter linkages for this construct are shown in the Table below.

Table: Insertion sites and promoter linkages in vCP1452

Insert	Insertion Site	Promoter
HIV1 MN gp120 + TM	C3	H6
HIV1 IIIB *gag* (+ pro)	C3	I3L
Pol3/Nef C term/Pol2/Nef central/Pol1	C5	H6
Vaccinia virus E3L	C6	endogenous
Vaccinia virus K3L	C6	H6

vCP300 is an ALVAC recombinant containing HIV gp120TM (MN), *gag*/pro (IIIB) (C3 locus), Nef (C6 locus), and Pol (C5 locus), as described in U.S. application Serial No. 08/417,210, incorporated herein by reference.

Plasmids for preparing these recombinants were prepared as follows:

Vaccinia H4L Expression Cassette Into ALVAC

pCPM6LDEL was generated by using primer pair H4A and H4B to amplify a 900 bp fragment from pBAMM11.6 (ALVAC 11.6kb *Bam*HI M fragment in pBSSK bector background), and primer pair H4C and H4D (SEQ ID NOS:) to amplify a 940 bp fragment from pBAMM11.6 (H4C 5'ACTACTAATTAGCTATAAAAACCCGGGATTAGTTTTTATTACTAACTAATTACTATACTG3') (H4D 5'ATCATCGGATCCTTTAATAATCTTATGAACTTTTATAAATATGAG3'). A fusion PCR reaction using the PCR products from the amplifications and primer pair H4A and H4D obrained an 1840 bp PCR fusion fragment which was then cloned into the T/A Cloning vector for sequence confirmation. The sequence was found to have a PCR deletion at position 8054. The 1840 bp fragment was removed from the T/A vector by digestion with *Bam*HI. The fragment was then cloned into the *Bam*HI digested pBSSK Δ*Eco*RI-*Sma*I vector. The deletion was repaired by digesting the construct with *Hind*III to remove a 250 bp fragment of the right arm and religating to obtain pCPM6LDEL.

placZH6H4Lreverse was digested with *Psp*AI and *Asp*700 resulting in a 1920bp fragment containing the H6 promoter and the 5' 1780bp of the H4L gene. The remaining 590bp of the H4L gene were generated using PCR amplification from the plasmid template placZH6H4Lreverse using primer pair H4A and H4B. The oligonucleotide sequences for primer pair H4A and H4B (SEQ ID NOS:) are:

Oligonucleotide Sequences

H4A 5'- ATCATCGAAGAGCTTCCGCTATCTGCATTAAAGTTT-3'

H4B 5'- ATCATCCCCGGGAAGCTTTTAGTTATTGAAATTAATCATATA-3'

The 590bp PCR fragment was gel purified and cloned into the TA Cloning vector (Invitrogen San Diego, CA. 92121) for sequence confirmation. The 590bp insert containing the 3' H4L sequences was excised from the TA vector by digestion with *Psp*AI and *Asp*700. The 1920bp and the 590bp fragments were directionally cloned into the *Psp*AI digested pCPM6LDEL plasmid vector (containing the deorfed ALVAC M6L insertion site) to generate the plasmid pM6LDELH6H4 containing the H6/H4L expression cassette flanked by ALVAC sequences at the M6L insertion site.

ALVAC pC8 insertion vector was generated as follows: PCR J36, containing the C8 ORF and flanking sequences, was generated using JP121 (CAT-CAT-GAG-CTC-ACT-TAT-TAC-ATC-CTA-CT) and JP122 (TAC-TAC-GGT-ACC-TTT-AAT-AAG-CAA-TCA-CT)
(SEQ ID NOS:) on ALVAC DNA. The resulting approximately 1.7 kb band was digested with *Asp*718/*Sac*I and ligated into *Asp*718/*Sac*I digested pBSSK+. After confirmation by sequence analysis, the resulting plasmid was designated pCPF85S3L. To remove most of the C8 ORF and introduce transcriptional and translational stops along with a MCS into pCPF85S3L, the plasmid was digested with *Sna*BI/*Hind*III and ligated to ~115bp PCR J618I *Sna*BI/*Hind*III fragment, yielding pC8. PCR J618I is a fusion PCR product of PCRs J616 and J617 using primers JP516 (TAG-GAA-GAT-ACG-TAT-TAT-TTT-ATA-C) and JP519 (ATC-

CCA-TTA-TGA-AAG-CTT-ATA-G). PCR J616 was generated using primers JP516 and JP517 (CTC-GAG-CTG-CAG-GAT-ATC-ATC-GAT-GGA-TCC-TTT-TTA-TAG-CTA-ATT-AGT-CAC-GTA-CCT-TTA-TCA-TTA-GTA-ACA-AAT) on plasmid pCPF85S3L. PCR J617 was generated using primers JP518 (GGA-TCC-ATC-GAT-GAT-ATC-CTG-CAG-CTC-GAG-TTT-TTA-TGA-CTA-GTT-AAT-CAC-GGC-CGC-TCA-ATA-TTG-TAT-TGG-ATG-GTT-AG) and JP519 on plasmid pCPF85S3L. Plasmid pC8, the C8 insertion plasmid, was confirmed by sequence analysis and contains a ~440bp left arm, a ~1162bp right arm, a MCS with unique *Bam*HI, *Cla*I, *Eco*RV, *Pst*I, and *Xho*I sites, flanked by both transcriptional and translational stop sequences.

From the plasmid pM6LDELH6H4, the 2.5 Kbp H6/H4 expression cassette was excised with *Sma*I, and the resulting 2.5 Kbp SmaI fragment was purified and inserted into the ALVAC pC8 insertion vector at the *Eco*RV site generating pC8H6H4.

K3L Expression Cassette

The K3L coding sequences were synthesized by PCR amplification using pSD407VC containing Copenhagen vaccinia HindIII K fragment as template, as described in U.S. Patent No. 5,378,457. The oligonucleotides MPSYN 763 and MPSYN 764 (SEQ ID NOS:) were used as primers for the PCR reaction.

MPSYN 763

5'-

CCCTCTAGATCGCGATATCCGTTAAGTTTGTATCGTAATGCTTGCATTTTGTTATTC

GT-3'

MPSYN 764 5'- CCCGAATTCATAAAAATTATTGATGTCTACA-3'

The approximately 325bp PCR fragment was digested with *Xba*I and *Eco*RI yielding a 315bp fragment. This 315bp fragment was purified by isolation from an agarose gel and ligated with *Xba*I and *Eco*RI digested pBSSK+ vector (from Stratagene LA Jolla, CA.). The nucleic acid sequence was confirmed directly from alkali denatured plasmid template as described in Hattori, M. and Sakaki, Y., 1986, using the modified T7 polymerase

(Tabor, S. and Richardson, C.C. 1987) and Sequenase (from U.S. Biochemicals Cleveland, OH.). This plasmid was designated pBS 763/764. Digesting pBS 763/764 with *Nru*I and *Xho*I, a 340bp fragment was isolated for cloning into the plasmid vector pMM154 containing a cassette with the vaccinia H6 promoter controlling an irrelevant gene in the NYVAC *tk* ⁻ insertion vector background, which was prepared by digestion with *Nru*I (partially) and *Xho*I, such that the 340bp fragment from pBS 763/764 containing the K3L gene could be directionally oriented next to the H6 promoter generating pMPTKH6K3L. The plasmid pMP42GPT containing the dominant selectable marker Eco gpt gene (Pratt D. and Subramani S. 1983) under the control of the Entomopox 42k promoter, was digested with *Sma*I and *Bam*HI to yield a 0.7 Kbp 42k-Eco gpt expression cassette. This 0.7 Kbp fragment was purified and ligated into *Sma*I and *Bam*HI cut pMPTKH6K3L generating the plasmid pMPTKH6K3Lgpt. This plasmid was digested with *Xho*I, generating a 1.2 Kbp fragment containing the H6/K3L and the 42k/Ecogpt expression cassette, which was then gel purified. The 1.2 Kbp XhoI fragment was inserted into the XhoI site of the ALVAC C6 insertion plasmid pC6L (described in U.S. Patent No. 5,494,807), generating pMPC6H6K3Lgpt.

<u>E3L/K3L ALVAC Expression Cassette</u>

The entire E3L gene is contained within a 2.3 Kbp *Eco*RI fragment isolated from pSD401VC, which contained a clone of the *Hind*III E fragment from Copenhagen vaccinia. The 2.3 Kbp *Eco*RI fragment was inserted into pMPC6H6K3Lgpt that had been partially digested with *Eco*RI, generating the plasmid pMPC6H6K3E3gpt. The plasmid pMPC6H6K3E3gpt was digested with *Xho*I and the resulting 6.8 Kbp vector fragment was purified and self-ligated, resulting in the plasmid pMPC6E3. The plasmid pMPTKH6K3L was digested with *Psp*AI and the resulting 560bp fragment containing the H6/K3L expression cassette was ligated into *Psp*AI digested

pMPC6E3 resulting in the plasmid construct pMPC6H6K3E3.

Construction of the H6-promoted FHV gB donor plasmid

The entire coding region of the Feline Herpesvirus 1 glycoprotein gB (FHV-1 gB) was obtained by digestion of pJCA079 (FHV gB coding region in which 5′ and 3′ T_5NT sequences were mutated to change the early transcriptional stop signal without affecting amino acid sequences; the I3L vaccinia promoter has been coupled to the 5′ end of the gB ORF; see Fig. 4, SEQ ID NO:) with *Pst*I and isolating a 3 Kbp fragment from an agarose gel. The purified *Pst*I fragment was cloned into an ALVAC C3 insertion plasmid (pVQH6CP3LSA) also digested with *Pst*I (the unique *Bam*HI site in pVQH6CP3LSA was previously inactivated by digestion with *Bam*HI, blunting the ends with Klenow polymerase and religation; pVQH6CP3LSA was obtained by digesting pVQH6CP3L, discussed in U.S. Patent No. 5,494,807, with *Not*I and *Nsi*I, from which a 6623 bp fragment was isolated and ligated to annealed oligonucleotides CP34 (5′GGCCGCGTCGACATGCA3′) and CP35 (5′TGTCGACGC3′) (SEQ ID NOS:). The resulting plasmid, pRAC5, was screened for proper orientation of the gB coding region with respect to the H6 promoter. To properly link the H6 promoter to the FHV gB initiation codon, an 800 bp PCR fragment was amplified from pJCA079 using oligonucleotides RG789 (SEQ ID NO:)(5′-TTTCATTATCGCGATATC-CGTTAAGTTTGTATCGTAATGTCCACTCGTGGCGATC-3′) and RG787 (SEQ ID NO:)(5′-GGAGGGTTTCAGAGGCAG-3′). This purified fragment was digested with *Nru*I/*Bam*HI and ligated into pRAC5 also digested with *Nru*I/*Bam*HI. The resulting plasmid was the FHV gB donor plasmid, pC3H6FHVB.

"String of Beads" Cassette

The "string of beads" expression cassette for the *nef* and *pol* CTL epitopes (H6/Pol 3/Nef C term/Pol 2/Nef central/Pol 1) was generated by PCR (polymerase chain reaction) as detailed below, using template pHXBD2 for pol epitopes and template 2-60-HIV.3 for Nef

epitopes. Initial assembly was in two parts: (1) H6(partial promoter)/Pol 3/Nef C term(Nef 2); (2) Pol 2/Nef central (Nef 1)/Pol 1 in pBSSK. These were combined, then moved to pBSH6-11 for the assembly of the entire H6 promoter, then the H6/HIV cassette was moved to a C5 insertion plasmid.

<u>(1) H6/Pol 3/Nef C term(Nef 2)</u>

A 230 bp fragment (A) was derived by PCR to obtain the H6 linkage and Pol3 using synthetic oligonucleotides MPSYN783 and MPSYN784 and template pHXBD2. pHXBD2 was derived at the NIH/NCI (Dr. Nancy Miller) from a recombinant phage library of *Xba*I digested DNA from HTLV-III infected H9 cells cloned in lambda-J1 (Shaw et al., 1994). This plasmid contains the entire proviral DNA sequence of the HIV IIIB isolate.

A 110 bp fragment (B) was derived by PCR to obtain Nef2 using oligonucleotides MPSYN785/MPSYN786 and template p2-60-HIV.3 (described in U.S. application Serial No. 417,210).

PCR fragments A and B were combined in a PCR as template to obtain a 300bp fragment containing H6 linkage/Pol3/Nef2 using external primers MPSYN783/MPSYN786 (SEQ ID NOS:). The 300bp fragment was digested with *Xho*I/*Hind*III and a 290 bp fragment was isolated and ligated with similarly digested pBSSK to generate pBS783/786. The sequence was confirmed.

<u>(2)Pol 2/Nef central (Nef 1)/Pol 1</u>

A 210 bp fragment (C) containing Pol2 was derived by PCR using synthetic oligonucleotides MPSYN787/MPSYN788 (SEQ ID NOS:) and template pHXBD2.

A 270 bp fragment (D) containing Nef1 was derived by PCR using synthetic oligonucleotides MPSYN789/MPSYN790 (SEQ ID NOS:) and template p2-60-HIV.3 (described in U.S. application Serial No. 08/417,210).

A 170 bp fragment (E) containing Pol1 was derived by PCR using primers MPSYN791/MPSYN792 (SEQ ID

NOS:) and template pHXBD2.

Fragments C and D were combined as template in a PCR for Pol 2/Nef 1 using external primers MPSYN787/MPSYN790 (SEQ ID NOS:) resulting in a 460 bp PCR product (C+D).

Fragments D and E were combined as template in a PCR for Nef 1/Pol 1 using external primers MPSYN789/MPSYN792 (SEQ ID NOS:), resulting in isolation of a 420 bp fragment (D+E).

Fragments (C+D) and (D+E) were combined as template in a PCR with external primers MPSYN787/MPSYN792 (SEQ ID NOS:) to obtain a 610 bp fragment containing Pol 2/Nef 1/Pol 1. This 610 bp fragment was digested with *Hind*III/*Pst*I. The resulting 590 bp fragment was ligated with pBSSK cut with *Hind*III/*Pst*I to generate pBS787/792. The sequence was confirmed.

MPSYN783: 5' CCC CTC GAG TCG CGA TAT CCG TTA AGT TTG TAT CGT AAT GCC ACT AAC AGA AGA AGC A 3' (58mer)

MPSYN784: 5' AAA TCT CCA CTC CAT CCT TGT TTT CAG ATT TTT AAA 3'(36 mer)

MPSYN785: 5' AAT CTG AAA ACA GGA ATG GAG TGG AGA TTT GAT TCT 3'(36 mer)

MPSYN786: 5' CCC AAG CTT ACA ATT TTT AAA ATA TTC AGG 3' (30 mer)

MPSYN787: 5' CCC AAG CTT ATG GCA ATA TTC CAA AGT AGC 3' (30 mer)

MPSYN788: 5' TGG AAA ACC TAC CAT GGT TGT AAG TCC CCA CCT CAA 3'(36 mer)

MPSYN789: 5' TGG GGA CTT ACA ACC ATG GTA GGT TTT CCA GTA ACA 3'(36 mer)

MPSYN790: 5' TAC AGT CTC AAT CAT TGG TAC TAG CTT GTA GCA CCA 3' (36 mer)

MPSYN791: 5' TAC AAG CTA GTA CCA ATG ATT GAG ACT GTA CCA GTA 3'(36 mer)

MPSYN792: 5' CCC CCT GCA GAA AAA TTA AGG CCC AAT TTT TGA AAT 3' (36 mer)

(SEQ ID NOS:)

Assembly of entire cassette:

A 590 bp *Hind*III/*Pst*I fragment was isolated from pBS787/792 and ligated with vector pBS783/786 cut with *Hind*III/*Pst*I to generate pBS783/792. pBS783/792 was cut with *Eco*RV and *Pst*I, to generate an 880 bp fragment which was then ligated with similarly digested vector pBSH6-1 to generate pBSH6PN. Plasmid pBSH6PN was digested with *Bam*HI and a 1060 bp fragment was isolated. pVQC5LSP1, a generic C5 donor plasmid, was digested with *Bam*HI and ligated with the 1060 bp fragment from pBSH6PN. The resulting plasmid, pMPC5H6PN, contains the HIV pol/nef "string of beads" cassette in the ALVAC C5 locus.

Example 3 - Expression studies

Example 3.1 - NYVAC Expression Results

Dishes containing confluent monolayers of cells were infected at a multiplicity of infection (moi) of 2. After incubation for specified time periods, cells were incubated in labeling medium for 1 hour. At the end of the incubation, cells were harvested for immunoprecipitation analysis as described (Harlow, E and Lane, D (1988) ; Langone, J. (1982)).

Cells were infected at an moi of 2 pfu/cell and incubated for specified time periods. At the appropriate time post-infection, cell lysates were prepared for RNA analysis. The medium was aspirated and cells were harvested. RNA was isolated and prepared using the TRI-Reagent (Molecular Research Center Inc. Cincinnati, OH. 45212) as per manufacture instructions and analyzed by slot blot. Radiolabelled DNA probes were used to detect specific RNA species.

The effect of vP1379 and vP1380 compared to the parental virus vP994 on the expression of HIV *env* truncated MN strain was studied by radiolabeling at specific times post-infection on CEF cells. IP analysis with monoclonal antibody against HIV *env* truncated MN strain (mAb K3A) revealed a significant increase in *de*

novo synthesis for vP1380 infected cells at early times post infection compared to either vP994 parental virus or vP1379. A similar trend is observed at late times post infection. IP analysis with rabbit anti H4L antiserum (provided by Dr. S. Shuman, Sloan-Kettering Institute, NY) show that only vP1380 infected cells expressed H4L product early in infection. Neither vP994 nor vP1379 infected cells expressed H4L early in infection. All samples show *de novo* synthesis of H4L late in infection, but expression rates are higher for vP1380 infected cells than for either vP994 or vP1379 infected cells. IP analysis of E3L product, a constitutive vaccinia protein, show that *de novo* synthesis occurs at a higher rate at all times post infection in vP1380 infected cells than in either vP994 or vP1379 infected cells.

These results indicate that vP1379 is a defective recombinant with a pattern of expression identical to the parental virus unlike vP1380 recombinant which expresses H4L at early and late times post-infection. This early H4 expression clearly correlates with the enhanced expression of the proteins under study (HIV *env* and E3L) at early times post-infection.

The following studies were conducted with vP1380 and vP994 since vP1379 does not express H4L product at early times post-infection.The rate of expression at different times post infection in HeLa cells (non permissive system) was studied by IP analysis. IP analysis with anti-H4L shows that vP1380 infected cells expressed H4L product at all times post-infection (3, 6, 24 and 48 Hrs.). No product was detected in vP994 infected cells at any time post infection. Sustained *de novo* synthesis is observed that increases with time. Analysis of HIV Env product shows that, although product expression levels are higher at all times for vP1380 infected cells vs. vP994's, the most significant difference is seen at late times, 24 and 48 Hrs., suggesting that expression of H4L must have an impact at

some level on expression of HIV Env product. Expression of E3L product is also increased in vP1380 infected cells compared to vP994.

Experiments performed on L929 cells gave similar results. The most significant difference was that expression rates of the H4L product at all times post infection was very low, however there was a dramatic difference in the *de novo* synthesis rate of HIV Env component. Differences in the rates of Env synthesis peaked at 24 hours with a 5 to 10 fold increase in vP1380 infected cells compared to vP994.

Since H4L product is an early transcription factor, it is of interest to determine if the results obtained at the expression level correlate with an increase in H4L message in vP1380 infected cells. RNA analysis by slot blots indicate that H4L message is detectable at all times post infection in vP1380 infected cells and achieved a steady state at 6 Hrs. post infection.

HIV Env message levels increase rapidly to steady state levels at 3 Hrs post infection and remained at those levels for all time points in vP1380 infected cells. On the other hand, vP994 infected cells show a peak of HIV *env* message at 6 hours post infection and a decline starting at 12 hours. E3L message in vP1380 infected cells is present at higher levels for all times post infection compared to vP994 infected cells. This pattern of RNA levels is consistent with the pattern of *de novo* synthesis rate at the protein level.

Mice were immunized by the intraperitoneal route on
day 0 and 28. Starting prior to the first immunization and at two week intervals following the immunization, mice were bled from the retroorbital plexus. Sera were prepared from the collected blood by standard clotting techniques and stored frozen at -20°C until use in kinetics ELISA for antibodies reactive to the HIV

envelope glycoprotein.

High doses of vP994 or vP1380 elicited similar levels of antibodies (Table below). However, at the lowest dose, $5X10^6$ pfu, only vP1380 was capable of generating HIV antibodies. Moreover, the level of antibodies induced by the low dose was comparable to the levels of antibodies elicited by the highest dose, $5X10^7$ pfu.

At a dose too low for vP994, lacking vaccinia H4 but identical in all other respects to vP1380, to elicit an antibody response, vP1380 induced antibody responses equivalent to those elicited by the highest doses tested. Thus, the overexpression of vaccinia H4L in NYVAC may result in increased potency for inducing humoral responses.

TABLE: Antibody responses to recombinant HIV-1 MN/BRU gp160.

			KINETICS (mOD/min) WEEKS							
VIRUS	DOSE	MOUSE	0	2	4	6	8	10	12	14
NYVAC	HI	a	0	1	0	1	2	2	1	2
		b	0	0	0	2	2	2	2	2
		c	0	0	0	2	1	1	1	1
vP994	HI	a	0	6	8	42	45	44	44	45
		b	0	1	1	34	42	35	24	23
		c	3	1	3	34	40	31	33	34
vP994	LO	a	0	1	2	4	3	3	5	6
		b	1	1	0	2	2	3	3	3
		c	1	0	1	14	16	17	12	13
vP1380	HI	a	2	8	39	41	49	47	52	50
		b	3	12	45	49	46	51	54	49
		c	1	7	35	42	41	43	40	39
vP1380	LO	a	1	2	3	49	45	47	46	44
		b	0	1	2	30	30	34	36	40
		c	0	3	14	54	48	51	51	54

Mice were inoculated during weeks 0 and 4.
vP994, HIV 1 MN gp140, noncleavable, secreted envelope glycoprotein.
vP1380, HIV 1 MN gp140 + vaccinia H4L transcription factor.
vCP125, HIV 1 MN gp160.
HI dose, $5X10^7$ pfu.
LO dose, $5X10^6$ pfu.

As discussed above, possibly, part of the enhanced levels in vP1380 are due to enhanced transcription and expression of viral specific products such as E3L, such that there is enhanced transcription and translation involved in expression in vP1380. There was more expression of the exogenous DNA and at more persistent levels in vP1380, in accordance with the invention wherein vectors obtain greater levels of expression and more persistent levels of expression. Enhanced expression profiles in the murine system provided enhanced immunogenicity in mice, as shown by vP1380 being more immunogenic in mice than vP994.

Another observation is that enhancement profiles are seen in restrictive early cells in the abortive early ALVAC recombinants herein, whereas the profiles were not observed in cells where there was productive replication, e.g., VERO or CEF, suggesting that the factor and the foreign DNA preferably should be expressed substantially co-temporally or contemporaneously, i.e., that preferably there should be co-expression at substantially the same time or stage, and that the time of expression, e.g., early, late, early and late, should be matched with the phenotype of the vector (e.g., abortive early, abortive late), i.e., that in a system in which viral replication is not impaired (a permissive system) or in a system in which replication is aborted at a time when expression is not matched with the phenotype of the vector may not obtain optimal expression. Thus, in an abortive early system such as ALVAC or NYVAC, one preferably expresses exogenous DNA and a transcriptional or transcriptional and translational factor early; in an abortive late system, one preferably expresses exogenous DNA and a transcriptional or transcriptional and translational factor late or early and late (as expression only early may be akin to expression in a permissive system, i.e., one may not necessarily obtain optimal expression).

Example 3.2 - ALVAC Expression Results

ALVAC-HIV Recombinants

Immunoprecipitation (IP) was used to provide a semi-quantitative comparison of the temporal expression of the HIV-I cassette contained in the ALVAC recombinants in MRC-5 infected cells. Heat inactivated sera from HIV patients was obtained and used for the IP as described in the methods. The antiserum will precipitate the 120 KDa env protein and the various cleavage products from the gag protein precursor. In the analysis of the IP data it is apparent that the ALVAC recombinants vCP1431A and vCP1437A containing the E3L/K3L cassette had a significant increase in the level of expression at all

times post infection when compared to the ALVAC recombinant vCP205 without the E3L/K3L cassette.

Interestingly vCP1431A and vCP1437A had similar expression profiles; insertion of H6/H4L into an ALVAC E3L/K3L background did not enhance expression above E3L/K3L, suggesting that vaccinia H4L is not necessarily functional in ALVAC; but, manipulation of ALVAC transcriptional factors would lead to enhanced expression. Although there are homologs of vaccinia transcriptional factors in canarypox, the requirements in canarypox may be biochemically different; but, these differences can be ascertained by the skilled artisan without undue experimentation from this disclosure and the knowledge in the art. Furthermore, the present invention provides *in vitro* systems for transcriptional analysis in canarypox or fowlpox using vaccinia virus.

RNA slot blots were used to evaluate temporal transcriptional expression in MRC-5 cells infected with the ALVAC recombinants vCP205 and vCP1431A and vCP1437A. In this analysis comparisons were made to the levels of mRNA transcribed from the HIV-I cassette encoding the env and gag proteins. ALVAC recombinants containing the E3L/K3L cassette (vCP1431A and vCP1437A) did not exhibit a significant increase in the level of mRNA for the env and gag genes above that of the ALVAC recombinant vCP205.

The previously discussed role E3L/K3L plays in the down regulation of PKR in vaccinia infected cells thereby modulating translation seems to be operative in the ALVAC recombinants containing the vaccinia E3L/K3L functions. The data has shown that translation is significantly enhanced in cells infected with ALVAC recombinants containing the E3L/K3L genes, while no significant increase in the level of transcription has been detected. This exemplifies the impact of E3L/K3L expression on translation efficiency in poxvirus infected cells.

Immunoprecipitation analyses were also

performed using radiolabeled lysates derived from CEF cells infected with ALVAC parental virus, ALVAC-MN120TMG (vCP205), ALVAC-MN120TMGNPst (vCP1433), vCP1452 and vCP300, as described previously (Taylor et al., 1990), with human serum derived from HIV-seropositive individuals (anti-HIV). The analysis confirmed the expression of the envelope sequences with a molecular weight of 120kDa and the Gag precursor protein with a molecular weight of 55kDa in the recombinants but not in the parental virus. However, vCP300 exhibits diminished expression in comparison to vCP1452, i.e., vCP1452 surprisingly demonstrates enhanced expression due to expression of transcription and/or translation factors, in accordance with the invention.

FAC scan analysis with the Human anti-HIV antibody demonstrated expression of gp120 on the surface of HeLa cells infected with ALVAC-MN120TMGNPst (vCP1433). No fluorescence was detected on cells infected with ALVAC parental virus.

Appropriate expression of the inserted HIV genes was further confirmed by immunoprecipitation analysis (using polyclonal serum pool from HIV infected individuals) performed on a radiolabelled lysate of MRC5 cells infected with vCP1433 or vCP1452. The analysis confirmed the expression of the envelope sequences with a molecular weight of 120KDa and the Gag precursor protein with a molecular weight of 55 KDa in vCP1452.

vCP1452 had enhanced expression on human cells in comparison to vCP1433 and vCP300. Indeed, enhanced expression was observed with the E3L/K3L translational factor in human and canine cells.

Preliminary immunogenicity studies in mice showed no evidence of enhanced immunogenicity by the E3L/K3L translational factor. This corresponds to no observed enhanced expression in murine cells.

Furthermore, in murine cells, the limiting factor of ALVAC expression is at the transcription level.

Accordingly, use of an appropriate transcription factor can overcome the inability to observe enhanced expression in the murine system. Thus, the origin of the cell may be an important factor in *in vitro* or *in vivo* applications of the invention (note H4 data above), as may be the nature of the vector, e.g., the phenotype of the vector (e.g., abortive, and when abortive such as abortive early, abortive late); but, appropriate selection of a cell and vector phenotype and of time of expression of factor(s) and foreign and/or exogenous DNA are within the ambit of the skilled artisan, from this disclosure and the knowledge in the art, without undue experimentation.

ALVAC-FHV gB Recombinants

Analysis of the expression for vCP1459, vCP1460 and vCP1464 was accomplished by immunoprecipitation analysis using a sheep anti-FHV gB polyclonal sera. Human MRC-5 cells were inoculated at an moi =5 at time 0, and then pulsed for 1 hour with ^{35}S labelled methionine at times 3, 6, 24, 48 and 72 h p.i. The precipitated protein was separated on SDS-PAGE gels. Autoradiographs of these IPs were scanned using a densitometer. The methods used provide a semi-quantitative analysis of FHV gB expression at the specific time points.

Results show that all recombinants express the proper sized full-length, glycosylated FHV gB polypeptide (apparent MW of approximately 115 kDa). However, recombinants vCP1460 and vCP1464 show significant increase in the amount of gB protein (about 5 times) compared to vCP1459. In addition, these expression levels persist even at 72 hr p.i. Thus, it appears that the expression of vaccinia E3L/K3L in ALVAC has a significant effect on the level and persistence of FHV gB expression.

Example 4 - Additional Vectors

Using the documents cited herein and the teaching herein, including in the foregoing Examples,

plasmid and naked DNA vectors, and additional viral vectors, including poxvirus, e.g., NYVAC, TROVAC, ALVAC, MVA, ts (temperature sensitive) mutants, or early (DNA$^-$) and late defective mutants, adenovirus, e.g., CAV such as CAV2, herpesvirus, e.g., Epstein Barr, are generated with enhanced transcription or translation or transcription and translation, e.g., by using H4L, vaccinia D6, vaccinia A7, vaccinia G8R, vaccinia A1L, vaccinia A2L, vaccinia H5R (VLTF-1, -2, -3, -4, P3, VLTF-X) E3L, K3L, VAI, EBER, sigma 3, TRBP, or combinations thereof to modify the vector to contain at least one transcritpion factor or at least one translation factor or at least one transcription factor and at least one translation factor; and accordingly, enhanced expression, of exogenous coding nucleic acid molecules (such exogenous coding nucleic acid molecules including from documents cited herein or as otherwise known in the art, or from applying those teachings in conjunction with teachings herein) is obtained.

Having thus described in detail preferred embodiments of the present invention, it is to be understood that the invention defined by the appended claims is not to be limited to particular details set forth in the above description as many apparent variations thereof are possible without departing from the spirit or scope of the present invention.

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WHAT IS CLAIMED IS:

1. A vector for enhanced expression of at least one first nucleic acid molecule in a cell having a particular phenotype, said vector modified to comprise the first nucleic acid molecule and at least one second nucleic acid molecule encoding a transcription factor or a transcription factor and a translation factor, wherein there is substantially co-temporal expression of the first and second nucleic acid molecules with respect to the phenotype of the cell, whereby expression of the second nucleic acid molecule enhances expression of the first nucleic acid molecule by enhancing transcription or transcription and translation.

2. The vector of claim 1 wherein the first nucleic acid molecule is operably linked to a first promoter and the second nucleic acid molecule is operably linked to a second promoter, and the first and second promoters are functional substantially co-temporally.

3. The vector of claim 2 wherein the first and second nucleic acid molecules are at different loci within the vector.

4. The vector of claim 2 wherein the first and second nucleic acid molecules are at the same locus within the vector.

5. The vector of claim 1 wherein the first nucleic acid molecule and the second nucleic acid molecule are operably linked to a promoter.

6. The vector of claim 1 wherein transcription factor is of poxvirus origin.

7. The vector of claim 6 wherein the transcription factor is from a vaccinia virus.

8. The vector of claim 7 wherein the transcription factor is from an open reading frame selected from the group consisting of H4L, D6, A7, G8R, A1L, A2L, H5R, and combinations thereof.

9. The vector of claim 1 wherein the second nucleic acid molecule is comprised of at least one

transcription factor and at least one translation factor.

10. The vector of claim 1 wherein the translation factor effects inhibition of eIF-2α phosphorylation or inhibition of PKR phosphorylation or otherwise sequesters dsRNA, increasing the effective concentration of dsRNA.

11. The vector of claim 10 wherein said at least one second molecule is selected from the group consisting of: a K3L open reading frame, an E3L open reading frame, a VAI RNA frame, an EBER RNA, a sigma 3 open reading frame, a TRBP open reading frame, and combinations thereof.

12. The vector of claim 1 wherein said first nucleic acid molecule encodes a molecule selected from the group consisting of an epitope of interest, a biological response modulator, a growth factor, a recognition sequence, a therapeutic gene and a fusion protein.

13. The vector of claim 1 which is a recombinant virus.

14. The vector of claim 13 which is a recombinant poxvirus.

15. A method for preparing a vector as claimed in claim 1 comprising modifying the vector to comprise the at least one second nucleic acid molecule, and optionally also modifying the vector to comprise the first nucleic acid molecule, so that there is substantially co-temporal expression of the first and second nucleic acid molecules with respect to the phenotype of the cell.

16. The method for claim 15 comprising operably linking the first nucleic acid molecule to a first promoter and the second nucleic acid molecule to a second promoter, wherein the first and second promoters are functional substantially co-temporally.

17. The method for claim 15 comprising operably linking the first and second nucleic acid

molecules to a promoter.

18. An immunological, vaccine or theraputic composition comprising the vector of claim 1 and a pharmaceutically acceptable carrier or diluent.

19. A method for generating an immunological or theraputic response in a host comprising administering to the host the composition of claim 18.

20. A method for increasing expression of at least one first nucleic acid molecule by a vector comprising the first nucleic acid molecule, wherein the expression is in a cell having a particular phenotype, and the method comprising modifying the vector to comprise at least one second nucleic acid molecule encoding a transcription factor or a transcription factor and a translation factor, wherein there is substantially co-temporal expression of the first and second nucleic acid molecules with respect to the phenotype of the cell, whereby expression of the second nucleic acid molecule enhances expression of the first nucleic acid molecule by enhancing transcription or transcription and translation.

21. A method for expressing a gene product *in vitro* comprising infecting, or transfecting, a suitable cell with a vector as claimed in claim 1.

22. A method for expressing the first nucleic acid molecule *in vivo* comprising administering the vector of claim 1 to a host.

23. vCP1452 or vCP1433.

FIG. 1

Fig. 1

Nucleotide sequence of the insert in vP1380 containing the mutagenized H4L orf and lacZ orf under the H6 promoter.

Characteristic	Position(s)
Left arm	1-798
Right arm	6636-7319
H6 promoter	C3307-3184 and C6495-6372
H4L orf	C3183-799
T to C mutations	C2836 and C2839
lacZ orf	C6371-3327

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   1 GGATCCTGCC GTTCCTATTC TAGACCAAAA ATTCGGTTTC ATGTTTTCGA AGCGGTGTTC
  61 TGCAACAAGT CGGGGATCGT GTTCTACATA TTTGGCGGCA TTATCCAGTA TCTGCCTATT
 121 GATCTTCATT TCGTTTTCGA TTCTGGCTAT TTCAAAATAA AATCCCGATG ATAGACCTCC
 181 AGACTTTATA ATTTCATCTA CGATGTTCAG CGCCGTAGTA ACTCTAATAA TATAGGCTGA
 241 TAAGCTAACA TCATACCCTC CTGTATATGT GAATATGGTA TGATTTTTGT CCATTACAAG
 301 CTCGGTTTTA ACTTTATTGC CTGTAATAAT TTCTCTCATC TGTAGGATAT CTATTTTTTT
 361 GTCATGCATT GCCTTCAAGA CGGGACGAAG AAACGTAATA TCCTCAATAA CGTTATCGTT
 421 TTCTACAATA ACTACATATT CTACCTTTTT ATTTTCTAAC TCGGTAAAAA AATTAGAATC
 481 CCATAGGGCT AAATGTCTAG CGATATTTCT TTTCGTTTCC TCTGTACACA TAGTGTTACA
 541 AAACCCTGAA AAGAAGTGAG TATACTTGTC ATCATTTCTA ATGTTTCCTC CAGTCCACTG
 601 TATAAACGCA TAATCCTTGT AATGATCTGG ATCATCCTTG ACTACCACAA CATTTCTTTT
 661 TTCTGGCATA ACTTCGTTGT CCTTTACATC ATCGAACTTC TGATCATTAA TATGCTCATG
 721 AACATTAGGA AATGTTTCTG ATGGAGGTCT ATCAATAACT GGCACAACAA TAACAGGAGT
 781 TTTCACCGCC GCCATTTAGT TATTGAAATT AATCATATAC AACTCTTTAA TACGAGTTAT
 841 ATTTTCGTCT ATCCATTGTT TCACATTTAC ATATTTCGAC AAAAAGATAT AAAATGCGTA
 901 TTCCAATGCT TCTCTGTTTA ATGAATTACT AAAATATACA AACACGTCAC TGTCTGGCAA
 961 TAAATGATAT CTTAGAATAT TGTAACAATT TATTTTGTAT TGCACATGTT CGTGATCTAT
1021 GAGTTCTTCT TCGAATGGCA TAGGATCTCC GAATCTGAAA ACGTATAAAT AGGAGTTAGA
1081 ATAATAATAT TTGAGAGTAT TGGTAATATA TAAACTCTTT AGCGGTATAA TTAGTTTTTT
1141 TCTCTCAATT TCTATTTTTA GATGTGATGG AAAAATGACT AATTTTGTAG CATTAGTATC
1201 ATGAACTCTA ATCAAAATCT TAATATCTTC GTCACACGTT AGCTCTTTGA AGTTTTTAAG
1261 AGATGCATCA GTTGGTTCTA CAGATGGAGT AGGTGCAACA ATTTTTTGTT CTACACATGT
1321 ATGTACTGGA GCCATTGTTT TAACTATAAT GGTGCTTGTA TCGAAAAACT TTAATGCAGA
1381 TAGCGGAAGC TCTTCGCCGC GACTTTCTAC ATCGTAATTG GGTTCTAACG CCGATCTCTG
1441 AATGGATACT AGTTTTCTAA GTTCTAATGT GATTCTCTGA AAATGTAAAT CCAATTCCTC
1501 CGGCATTATA GATGTGTATA CATCGGTAAA TAAAACTATA GTATCCAACG ATCCCTTCTC
1561 GCAAATTCTA GTCTTAACCA AAAAATCGTA TATAACCACG GAGATGGCGT ATTTAAGAGT
1621 GGATTCTTCT ACCGTTTTGT TCTTGGATGT CATATAGGAA ACTATAAAGT CCGCACTACT
1681 GTTAAGAATG ATTACTAACG CAACTATATA GTTCAAATTA AGCATTTTGG AAACATAAAA
1741 TAACTCTGTA GACGATACTT GACTTTCGAA TAAGTTTGCA GACAAACGAA GAAAGAACAG
1801 ACCTCTCTTA ATTTCAGAAG AAAACTTTTT TTCGTATTCC TGACGTCTAG AGTTTATATC
1861 AATAAGAAAG TTAAGAATTA GTCGGTTAAT GTTGTATTTC ATTACCCAAG TTTGAGATTT
1921 CATAATATTA TCAAAAGACA TGATAATATT AAAGATAAAG CGCTGACTAT GAACGAAATA
1981 GCTATATGGT TCGCTCAAAA ATATAGTCTT GTTAAACGTG GAAACGATAA CTGTATTTTT
2041 AATCACGTCA GCGGCATCTA AATTAAATAT AGGTATATTT ATTCCACACA CTCTACAATA
2101 TGCCACACCA TCTTCATAAT AAATAAATTC GTTAGCAAAA TTATTAATTT TAGTGAAATA
2161 GTTAGCGTCA ACTTTCATAG CTTCCTTCAA TCTAATTTGA TGCTCACACG GTGCGAATTC
2221 TACTCTAACA TCCCTTTTCC ATGCCTCAGG TTCATCGATC TCTATAATAT CTAGTTTTTT
2281 GCGTTTCACA AACACAGGCT CGTCTCTCGC GATGAGATCT GTATAGTAAC TATGTAAATG
2341 ATAACTAGAT AGAAAGATGT AGCTATATAG ATGACGATCC TTTAAGAGAG GTATAATAAC
2401 TTTACCCCAA TCAGATAGAC TGTTGTTATG GTCTTCGGAA AAAGAATTTT TATAAATTTT
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F1G. 1 (cont'd)

2461 TCCAGTATTT TCCAAATATA CGTACTTAAC ATCTAAAAAA TCCTTAATGA TAATAGGAAT
2521 GGATAATCCG TCTATTTTAT AAAGAAATAC ATATCGCACA TTATACTTTT TTTTGGAAAT
2581 GGGAATACCG ATGTGTCTAC ATAAATATGC AAAGTCTAAA TATTTTTTAG AGAATCTTAG
2641 TTGGTCCAAA TTCTTTTCCA AGTACGGTAA TAGATTTTTC ATATTGAACG GTATCTTCTT
2701 AATCTCTGGT TCTAGTTCCG CATTAAATGA TGAAACTAAG TCACTATTTT TATAACTAAC
2761 GATTACATCA CCTCTAACAT CATCATTTAC CAGAATACTG ATCTTCTTTT GTCGTAAATA
2821 CATGTCTAAT GTGTTGAAGA AAAGATCATA CAAGTTATAC GTCATTTCAT CTGTGGTATT
2881 CTTGTCATTG AAGGATAAAC TCGTACTAAT CTCTTCTTTA ACAGCCTGTT CAAATTTATA
2941 TCCTATATAC GAAAAAATAG CAACCAGTGT TTGATCATCC GCGTCAATAT TCTGTTCTAT
3001 CGTAGTGTAT AACAATCGTA TATCTTCTTC TGTGATAGTC GATACGTTAT AAAGGTTGAT
3061 AACGAAAATA TTTTTATTTC GTGAAATAAA GTCATCGTAG GATTTTGGAC TTATATTCGC
3121 GTCTAGTAGA TATGCTTTTA TTTTTGGAAT GATCTCAATT AGAATAGTCT CTTTAGAGTC
3181 CATTACGATA CAAACTTAAC GGATATCGCG ATAATGAAAT AATTTATGAT TATTTCTCGC
3241 TTTCAATTTA ACACAACCCT CAAGAACCTT TGTATTTATT TTCACTTTTT AAGTATAGAA
3301 TAAAGAAGCT TCCCGGGGGA TCCTTATTTT TGACACCAGA CCAACTGGTA ATGGTAGCGA
3361 CCGGCGCTCA GCTGGAATTC CGCCGATACT GACGGGCTCC AGGAGTCGTC GCCACCAATC
3421 CCCATATGGA AACCGTCGAT ATTCAGCCAT GTGCCTTCTT CCGCGTGCAG CAGATGGCGA
3481 TGGCTGGTTT CCATCAGTTG CTGTTGACTG TAGCGGCTGA TGTTGAACTG GAAGTCGCCG
3541 CGCCACTGGT GTGGGCCATA ATTCAATTCG CGCGTCCCGC AGCGCAGACC GTTTTCGCTC
3601 GGGAAGACGT ACGGGGTATA CATGTCTGAC AATGGCAGAT CCCAGCGGTC AAAACAGGCG
3661 GCAGTAAGGC GGTCGGGATA GTTTTCTTGC GGCCCTAATC CGAGCCAGTT TACCCGCTCT
3721 GCTACCTGCG CCAGCTGGCA GTTCAGGCCA ATCCGCGCCG GATGCGGTGT ATCGCTCGCC
3781 ACTTCAACAT CAACGGTAAT CGCCATTTGA CCACTACCAT CAATCCGGTA GGTTTTCCGG
3841 CTGATAAATA AGGTTTTCCC CTGATGCTGC CACGCGTGAG CGGTCGTAAT CAGCACCGCA
3901 TCAGCAAGTG TATCTGCCGT GCACTGCAAC AACGCTGCTT CGGCCTGGTA ATGGCCCGCC
3961 GCCTTCCAGC GTTCGACCCA GGCGTTAGGG TCAATGCGGG TCGCTTCACT TACGCCAATG
4021 TCGTTATCCA GCGGTGCACG GGTGAACTGA TCGCGCAGCG GCGTCAGCAG TTGTTTTTTA
4081 TCGCCAATCC ACATCTGTGA AAGAAAGCCT GACTGGCGGT TAAATTGCCA ACGCTTATTA
4141 CCCAGCTCGA TGCAAAAATC CATTTCGCTG GTGGTCAGAT GCGGGATGGC GTGGGACGCG
4201 GCGGGGAGCG TCACACTGAG GTTTTCCGCC AGACGCCACT GCTGCCAGGC GCTGATGTGC
4261 CCGGCTTCTG ACCATGCGGT CGCGTTCGGT TGCACTACGC GTACTGTGAG CCAGAGTTGC
4321 CCGGCGCTCT CCGGCTGCGG TAGTTCAGGC AGTTCAATCA ACTGTTTACC TTGTGGAGCG
4381 ACATCCAGAG GCACTTCACC GCTTGCCAGC GGCTTACCAT CCAGCGCCAC CATCCAGTGC
4441 AGGAGCTCGT TATCGCTATG ACGGAACAGG TATTCGCTGG TCACTTCGAT GGTTTGCCCG
4501 GATAAACGGA ACTGGAAAAA CTGCTGCTGG TGTTTTGCTT CCGTCAGCGC TGGATGCGGC
4561 GTGCGGTCGG CAAAGACCAG ACCGTTCATA CAGAACTGGC GATCGTTCGG CGTATCGCCA
4621 AAATCACCGC CGTAAGCCGA CCACGGGTTG CCGTTTTCAT CATATTTAAT CAGCGACTGA
4681 TCCACCCAGT CCCAGACGAA GCCGCCCTGT AAACGGGGAT ACTGACGAAA CGCCTGCCAG
4741 TATTTAGCGA AACCGCCAAG ACTGTTACCC ATCGCGTGGG CGTATTCGCA AAGGATCAGC
4801 GGGCGCGTCT CTCCAGGTAG CGAAAGCCAT TTTTTGATGG ACCATTTCGG CACAGCCGGG
4861 AAGGGCTGGT CTTCATCCAC GCGCGCGTAC ATCGGGCAAA TAATATCGGT GGCCGTGGTG
4921 TCGGCTCCGC CGCCTTCATA CTGCACCGGG CGGGAAGGAT CGACAGATTT GATCCAGCGA
4981 TACAGCGCGT CGTGATTAGC GCCGTGGCCT GATTCATTCC CCAGCGACCA GATGATCACA
5041 CTCGGGTGAT TACGATCGCG CTGCACCATT CGCGTTACGC GTTCGCTCAT CGCCGGTAGC
5101 CAGCGCGGAT CATCGGTCAG ACGATTCATT GGCACCATGC CGTGGGTTTC AATATTGGCT
5161 TCATCCACCA CATACAGGCC GTAGCGGTCG CACAGCGTGT ACCACAGCGG ATGGTTCGGA
5221 TAATGCGAAC AGCGCACGGC GTTAAAGTTG TTCTGCTTCA TCAGCAGGAT ATCCTGCACC
5281 ATCGTCTGCT CATCCATGAC CTGACCATGC AGAGGATGAT GCTCGTGACG GTTAACGCCT
5341 CGAATCAGCA ACGGCTTGCC GTTCAGCAGC AGCAGACCAT TTTCAATCCG CACCTCGCGG
5401 AAACCGACAT CGCAGGCTTC TGCTTCAATC AGCGTGCCGT CGGCGGTGTG CAGTTCAACC
5461 ACCGCACGAT AGAGATTCGG GATTTCGGCG CTCCACAGTT TCGGGTTTTC GACGTTCAGA
5521 CGTAGTGTGA CGCGATCGGC ATAACCACCA CGCTCATCGA TAATTTCACC GCCGAAAGGC
5581 GCGGTGCCGC TGGCGACCTG CGTTTCACCC TGCCATAAAG AAACTGTTAC CCGTAGGTAG
5641 TCACGCAACT CGCCGCACAT CTGAACTTCA GCCTCCAGTA CAGCGCGGCT GAAATCATCA
5701 TTAAAGCGAG TGGCAACATG GAAATCGCTG ATTTGTGTAG TCGGTTTATG CAGCAACGAG
5761 ACGTCACGGA AAATGCCGCT CATCCGCCAC ATATCCTGAT CTTCCAGATA ACTGCCGTCA
5821 CTCCAACGCA GCACCATCAC CGCGAGGCGG TTTTCTCCGG CGCGTAAAAA TGCGCTCAGG
5881 TCAAATTCAG ACGGCAAACG ACTGTCCTGG CCGTAACCGA CCCAGCGCCC GTTGCACCAC
5941 AGATGAAACG CCGAGTTAAC GCCATCAAAA ATAATTCGCG TCTGGCCTTC CTGTAGCCAG
6001 CTTTCATCAA CATTAAATGT GAGCGAGTAA CAACCCGTCG GATTCTCCGT GGGAACAAAC
6061 GGCGGATTGA CCGTAATGGG ATAGGTTACG TTGGTGTAGA TGGGCGCATC GTAACCGTGC
6121 ATCTGCCAGT TTGAGGGGAC GACGACAGTA TCGGCCTCAG GAAGATCGCA CTCCAGCCAG

Fig. 1 (cont'd)

6181 CTTTCCGGCA CCGCTTCTGG TGCCGGAAAC CAGGCAAAGC GCCATTCGCC ATTCAGGCTG
6241 CGCAACTGTT GGGAAGGGCG ATCGGTGCGG GCCTCTTCGC TATTACGCCA GCTGGCGAAA
6301 GGGGGATGTG CTGCAAGGCG ATTAAGTTGG GTAACGCCAG GGTTTTCCCA GTCACGACGT
6361 TGTAAAACCA TTACGATACA AACTTAACGG ATATCGCGAT AATGAAATAA TTTATGATTA
6421 TTTCTCGCTT TCAATTTAAC ACAACCCTCA AGAACCTTTG TATTTATTTT CACTTTTTAA
6481 GTATAGAATA AAGAACCCGG GAAGCTTGTC TAGCTGGTGC TGAGTTTCTA CGTGAGTTGA
6541 TTCGTCTCTT GCGTGCCTCT CGTGATCCAA TTGTCCCGAG ATATTCTCTT CTCTTCCGGT
6601 GCTTCTTATG AAACTTTCCC TGACGGTGGC GTTTTAAAGT TACAAACAAC TAGGAAATTG
6661 GTTTATGATG TATAATTTTT TTAGTTTTTA TAGATTCTTT ATTCTATACT TAAAAAATGA
6721 AAATAAATAC AAAGGTTCTT GAGGGTTGTG TTAAATTGAA AGCGAGAAAT AATCATAAAT
6781 TATTTCATTA TCGCGATATC CGTTAAGTTT GTATCGTAAT GGCGTGGTCA ATTACAAATA
6841 AAGCGGATAC TAGTAGCTTC ACAAAGATGG CTGAAATCAG AGCTCATCTA AAAAATAGCG
6901 CTGAAAATAA AGATAAAAAC GAGGATATTT TCCCGGAAGA TGTAATAATT CCATCTACTA
6961 AGCCCAAAAC CAAACGAGCC ACTACTCCTC GTAAACCAGC GGCTACTAAA AGATCAACCA
7021 AAAAGGAGGA AGTGGAAGAA GAAGTAGTTA TAGAGGAATA TCATCAAACA ACTGAAAAAA
7081 ATTCTCCATC TCCTGGAGTC GGCGACATTG TAGAAAGCGT GGCTGCTGTA GAGCTCGATG
7141 ATAGCGACGG GGATGATGAA CCTATGGTAC AAGTTGAAGC TGGTAAAGTA AATCATAGTG
7201 CTAGAAGCGA TCTTTCTGAC CTAAAGGTGG CTACCGACAA TATCGTTAAA GATCTTAAGA
7261 AAATTATTAC TAGAATCTCT GCAGTATCGA CGGTTCTAGA GGATGTTCAA GCAGGATCC

Fig. 2

Fig.2

Nucleotide Sequence of the ALVAC C8 Insertion site containing the H6/H4L expression cassette

Characteristic	Position(s)bp
Left Arm	1-487
Right Arm	3016-4225
H6 Promoter	495-618
H4L orf	619-3003

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   1 GAGCTCACTT ATTACATCCT ACTGACTATA TACAGCGAAT TAACCATAGG CGTAATTGTA
  61 CAGAAACCAG GAAATTATTA CCGCCTTTTA TAAGAAGTAT TAATAAAACA TGTAGCGTAT
 121 GTCTAGAAAG AATATACGAA AAAGAAATAA ATAAACAATA TTTCGGTATT TTACCAAATT
 181 GTAAACACGT GTTTTGTTTT TACTGTATAC AACGTTGGAT GTCTATAATA AAAGGTACGG
 241 ATACCGAAGG TACATGTCCT GTATGTAGAA CAGTTTCTGT ATTTATAGTG CCTAATAGGT
 301 ACTGGATAGA CGATAAATAT GAAAAGAGAT TAATTATAAA TAAATATAAG AATGACAGAA
 361 AGACTTATAA AGCGTTTAAA CATTATATAG GAAGATACGT ATTATTTTAT ACAGTAAACA
 421 ACAGTTTATT TGTTACTAAT GATTAAGGTA CGTGACTAAT TAGCTATAAA AAGGATCCAT
 481 CGATGATGGG AAGCTTCTTT ATTCTATACT TAAAAAGTGA AAATAAATAC AAAGGTTCTT
 541 GAGGGTTGTG TTAAATTGAA AGCGAGAAAT AATCATAAAT TATTTCATTA TCGCGATATC
 601 CGTTAAGTTT GTATCGTAAT GGACTCTAAA GAGACTATTC TAATTGAGAT CATTCCAAAA
 661 ATAAAAGCAT ATCTACTAGA CGCGAATATA AGTCCAAAAT CCTACGATGA CTTTATTTCA
 721 CGAAATAAAA ATATTTTCGT TATCAACCTT TATAACGTAT CGACTATCAC AGAAGAAGAT
 781 ATACGATTGT TATACACTAC GATAGAACAG AATATTGACG CGGATGATCA AACACTGGTT
 841 GCTATTTTTT CGTATATAGG ATATAAATTT GAACAGGCTG TTAAAGAAGA GATTAGTACG
 901 AGTTTATCCT TCAATGACAA GAATACCACA GATGAAATGA CGTATAACTT GTATGATCTT
 961 TTCTTCAACA CATTAGACAT GTATTTACGA CAAAAGAAGA TCAGTATTCT GGTAAATGAT
1021 GATGTTAGAG GTGATGTAAT CGTTAGTTAT AAAAATAGTG ACTTAGTTTC ATCATTTAAT
1081 GCGGAACTAG AACCAGAGAT TAAGAAGATA CCGTTCAATA TGAAAAATCT ATTACCGTAC
1141 TTGGAAAAGA ATTTGGACCA ACTAAGATTC TCTAAAAAAT ATTTAGACTT TGCATATTTA
1201 TGTAGACACA TCGGTATTCC CATTTCCAAA AAAAAGTATA ATGTGCGATA TGTATTTCTT
1261 TATAAAATAG ACGGATTATC CATTCCTATT ATCATTAAGG ATTTTTTAGA TGTTAAGTAC
1321 GTATATTTGG AAAATACTGG AAAAATTTAT AAAAATTCTT TTTCCGAAGA CCATAACAAC
1381 AGTCTATCTG ATTGGGGTAA AGTTATTATA CCTCTCTTAA AGGATCGTCA TCTATATAGC
1441 TACATCTTTC TATCTAGTTA TCATTTACAT AGTTACTATA CAGATCTCAT CGCGAGAGAC
1501 GAGCCTGTGT TTGTGAAACG CAAAAAACTA GATATTATAG AGATCGATGA ACCTGAGGCA
1561 TGGAAAAGGG ATGTTAGAGT AGAATTCGCA CCGTGTGAGC ATCAAATTAG ATTGAAGGAA
1621 GCTATGAAAG TTGACGCTAA CTATTTCACT AAAATTAATA ATTTTGCTAA CGAATTTATT
1681 TATTATGAAG ATGGTGTGGC ATATTGTAGA GTGTGTGGAA TAAATATACC TATATTTAAT
1741 TTAGATGCCG CTGACGTGAT TAAAAATACA GTTATCGTTT CCACGTTTAA CAAGACTATA
1801 TTTTTGAGCG AACCATATAG CTATTTCGTT CATAGTCAGC GCTTTATCTT TAATATTATC
1861 ATGTCTTTTG ATAATATTAT GAAATCTCAA ACTTGGGTAA TGAAATACAA CATTAACCGA
1921 CTAATTCTTA ACTTTCTTAT TGATATAAAC TCTAGACGTC AGGAATACGA AAAAAAGTTT
1981 TCTTCTGAAA TTAAGAGAGG TCTGTTCTTT CTTCGTTTGT CTGCAAACTT ATTCGAAAGT
2041 CAAGTATCGT CTACAGAGTT ATTTTATGTT TCCAAAATGC TTAATTTGAA CTATATAGTT
2101 GCGTTAGTAA TCATTCTTAA CAGTAGTGCG GACTTTATAG TTTCCTATAT GACATCCAAG
2161 AACAAAACGG TAGAAGAATC CACTCTTAAA TACGCCATCT CCGTGGTTAT ATACGATTTT
2221 TTGGTTAAGA CTAGAATTTG CGAGAAGGGA TCGTTGGATA CTATAGTTTT ATTTACCGAT
2281 GTATACACAT CTATAATGCC GGAGGAATTG GATTTACATT TTCAGAGAAT CACATTAGAA
2341 CTTAGAAAAC TAGTATCCAT TCAGAGATCG GCGTTAGAAC CCAATTACGA TGTAGAAAGT
2401 CGCGGCGAAG AGCTTCCGCT ATCTGCATTA AAGTTTTTCG ATACAAGCAC CATTATAGTT
2461 AAAACAATGG CTCCAGTACA TACATGTGTA GAACAAAAAA TTGTTGCACC TACTCCATCT
2521 GTAGAACCAA CTGATGCATC TCTTAAAAAC TTCAAAGAGC TAACGTGTGA CGAAGATATT
2581 AAGATTTTGA TTAGAGTTCA TGATACTAAT GCTACAAAAT TAGTCATTTT TCCATCACAT
2641 CTAAAAATAG AAATTGAGAG AAAAAAACTA ATTATACCGC TAAAGAGTTT ATATATTACC
2701 AATACTCTCA AATATTATTA TTCTAACTCC TATTTATACG TTTTCAGATT CGGAGATCCT
2761 ATGCCATTCG AAGAAGAACT CATAGATCAC GAACATGTGC AATACAAAAT AAATTGTTAC
2821 AATATTCTAA GATATCATTT ATTGCCAGAC AGTGACGTGT TTGTATATTT TAGTAATTCA
2881 TTAAACAGAG AAGCATTGGA ATACGCATTT TATATCTTTT TGTCGAAATA TGTAAATGTG
2941 AAACAATGGA TAGACGAAAA TATAACTCGT ATTAAAGAGT TGTATATGAT TAATTTCAAT
3001 AACTAAAAGC TTCCCATCCT GCAGCTCGAG TTTTTATGAC TAGTTAATCA CGGCCGCTCA
```

Fig. 2 (cont'd)

3061 ATATTGTATT GGATGGTTAG AGATCAAAGG ATACAAGATA ACTGGGCTCA TTTCAGCTTT
3121 ACATTCATCC CTATAAGCTT TCATAATGGG ATTTTTCTCC ATAATGTCAA AATCACTTTG
3181 GATATATTCA AAATTTTCTA CAAAATGTTT TGGTTGTTCT GAGCTAAACA CGATGTTAGA
3241 TATTAATAAC TTTGCTATCT CAAGACCTTC TGAAGTATCA ACTTTGATAT TGGAAAGAGG
3301 TGTAAAATAA GGTGATGAAG CGATTGTTGT ATCTGCACAG AATGTTAACA GTATATCTAC
3361 TAATTCTACA TTTCCATCTG TCACAGCATG CCATAGAGGA GTATTCCAGT ACCTGTCCTT
3421 AGCATTTATA TCAGCACCGA ATTCCAAAAG CATAATAGTT ATCTTTACAG ATCCTATACA
3481 CACAGCATAA TGCAAAGGAG TCATCCTATG GCTATCTTTA ACGTTAGTAT ATGCTCCAGC
3541 TAGAAGTAAT TGCTCTATTA TCTCCATGTT TTCAGATTTA ACAGCATAAT GCAATGGATA
3601 CATATATCCT CTGTAACCAT AATTTATACT CGATCCAGCT TTTAGTAACA TACTCACAAT
3661 TTCCAAATTT TCTCTCTTTA TAGCCTCGAT TATGGGATGA TTTTCCCTGT ACTCATTGTC
3721 AACATCAGCG TTATACTCCA GAAGTAACTT TACAATTTCC ACATTCTCTA TAGAGACAGC
3781 ATACTGGAGT GGAGTCTTTA CTTTGTAGTC CTCATATGTA TCCACATTAG CGCCATGATC
3841 CAACAAGAGT TTCACCAGAT CTATGTTCTG AACTTTGACA GCTCTATGCA ACGGAGAAGA
3901 TACTTGTTCG CTAGATATAT CAGGATCAGC TCCTGCTAAC AATAGAGCTT TGGCTATTTC
3961 AAATTTTTCA TTTTCTACAG CACAATGAAG GGGTGAGCAG CCATAATCGT TGAATACGTC
4021 CAGGTTAATG CCGGTTTTCA CAATATCTAG CACGCTAGAC AGAGATCCAG ATTCAATAGC
4081 TTCGAATAAG TATGCCTCCA TTTTGTGTAA TAGTAGTAAG TAATAATTTT CTGAAGAAAC
4141 TACTAACTTA CCGAGCTATA GTAGATAGTT ATAATTTCAT TTTTTTACAA GTAGTATCAC
4201 ATAGTGATTG CTTATTAAAG GTACC

Fig. 3

Fig. 3

Nucleotide sequence of the ALVAC C6 insertion site containing the H6 / K3L and E3L expression cassette.

Characteristic	Position(s)
Left Arm	1-385
Right Arm	3273-4434
K3L orf	C727-464
H6 Promoter	C850-728
E3L	C2758-2188

1	GAGCTCGCGG	CCGCCTATCA	AAAGTCTTAA	TGAGTTAGGT	GTAGATAGTA	TAGATATTAC
61	TACAAAGGTA	TTCATATTTC	CTATCAATTC	TAAAGTAGAT	GATATTAATA	ACTCAAAGAT
121	GATGATAGTA	GATAATAGAT	ACGCTCATAT	AATGACTGCA	AATTTGGACG	GTTCACATTT
181	TAATCATCAC	GCGTTCATAA	GTTTCAACTG	CATAGATCAA	AATCTCACTA	AAAAGATAGC
241	CGATGTATTT	GAGAGAGATT	GGACATCTAA	CTACGCTAAA	GAAATTACAG	TTATAAATAA
301	TACATAATGG	ATTTTGTTAT	CATCAGTTAT	ATTTAACATA	AGTACAATAA	AAAGTATTAA
361	ATAAAAATAC	TTACTTACGA	AAAAATGACT	AATTAGCTAT	AAAAACCCAG	ATCTCTCGAG
421	GTCGACGGTA	TCGATAAGCT	TGATATCGAA	TTCATAAAAA	TTATTGATGT	CTACACATCC
481	TTTTGTAATT	GACATCTATA	TATCCTTTTG	TATAATCAAC	TCTAATCACT	TTAACTTTTA
541	CAGTTTTCCC	TACCAGTTTA	TCCCTATATT	CAACATATCT	ATCCATATGC	ATCTTAACAC
601	TCTCTGCCAA	GATAGCTTCA	GAGTGAGGAT	AGTCAAAAAG	ATAAATGTAT	AGAGCATAAT
661	CCTTCTCGTA	TACTCTGCCC	TTTATTACAT	CGCCCGCATT	GGGCAACGAA	TAACAAAATG
721	CAAGCATACG	ATACAAACTT	AACGGATATC	GCGATAATGA	AATAATTTAT	GATTATTTCT
781	CGCTTTCAAT	TTAACACAAC	CCTCAAGAAC	CTTTGTATTT	ATTTTCACTT	TTTAAGTATA
841	GAATAAAGAA	AGCTCTAATT	AATTAATGAA	CAGATTGTTT	CGTTTTCCCC	TTGGCGTATC
901	ACTAATTAAT	TAACCCGGGC	TGCAGCTCGA	GGAATTCAAC	TATATCGACA	TATTTCATTT
961	GTATACACAT	AACCATTACT	AACGTAGAAT	GTATAGGAAG	AGATGTAACG	GGAACAGGGT
1021	TTGTTGATTC	GCAAACTATT	CTAATACATA	ATTCTTCTGT	TAATACGTCT	TGCACGTAAT
1081	CTATTATAGA	TGCCAAGATA	TCTATATAAT	TATTTTGTAA	GATGATGTTA	ACTATGTGAT
1141	CTATATAAGT	AGTGTAATAA	TTCATGTATT	TCGATATATG	TTCCAACTCT	GTCTTTGTGA
1201	TGTCTAGTTT	CGTAATATCT	ATAGCATCCT	CAAAAAATAT	ATTCGCATAT	ATTCCCAAGT
1261	CTTCAGTTCT	ATCTTCTAAA	AAATCTTCAA	CGTATGGAAT	ATAATAATCT	ATTTTACCTC
1321	TTCTGATATC	ATTAATGATA	TAGTTTTTGA	CACTATCTTC	TGTCAATTGA	TTCTTATTCA
1381	CTATATCTAA	GAAACGGATA	GCGTCCCTAG	GACGAACTAC	TGCCATTAAT	ATCTCTATTA
1441	TAGCTTCTGG	ACATAATTCA	TCTATTATAC	CAGAATTAAT	GGGAACTATT	CCGTATCTAT
1501	CTAACATAGT	TTTAAGAAAG	TCAGAATCTA	AGACCTGATG	TTCATATATT	GGTTCATACA
1561	TGAAATGATC	TCTATTGATG	ATAGTGACTA	TTTCATTCTC	TGAAAATTGG	TAACTCATTC
1621	TATATATGCT	TTCCTTGTTG	ATGAAGGATA	GAATATACTC	AATAGAATTT	GTACCAACAA
1681	ACTGTTCTCT	TATGAATCGT	ATATCATCAT	CTGAAATAAT	CATGTAAGGC	ATACATTTAA
1741	CAATTAGAGA	CTTGTCTCCT	GTTATCAATA	TACTATTCTT	GTGATAATTT	ATGTGTGAGG
1801	CAAATTTGTC	CACGTTCTTT	AATTTTGTTA	TAGTAGATAT	CAAATCCAAT	GGAGCTACAG
1861	TTCTTGGCTT	AAACAGATAT	AGTTTTTCTG	GAACAAATTC	TACAACATTA	TTATAAAGGA
1921	CTTTGGGTAG	ATAAGTGGGA	TGAAATCCTA	TTTTAATTAA	TGCTATCGCA	TTGTCCTCGT
1981	GCAAATATCC	AAACGCTTTT	GTGATAGTAT	GGCATTCATT	GTCTAGAAAC	GCTCTACGAA
2041	TATCTGTGAC	AGATATCATC	TTTAGAGAAT	ATACTAGTCG	CGTTAATAGT	ACTACAATTT
2101	GTATTTTTTA	ATCTATCTCA	ATAAAAAAAT	TAATATGTAT	GATTCAATGT	ATAACTAAAC
2161	TACTAACTGT	TATTGATAAC	TAGAATCAGA	ATCTAATGAT	GACGTAACCA	AGAAGTTTAT
2221	CTACTGCCAA	TTTAGCTGCA	TTATTTTTAG	CATCTCGTTT	AGATTTTCCA	TCTGCCTTAT
2281	CGAATACTCT	TCCGTCGATG	TCTACACAGG	CATAAAATGT	AGGAGAGTTA	CTAGGCCCAA
2341	CTGATTCAAT	ACGAAAAGAC	CAATCTCTCT	TAGTTATTTG	GCAGTACTCA	TTAATAATGG
2401	TGACAGGGTT	AGCATCTTTC	CAATCAATAA	TTTTTTTAGC	CGGAATAACA	TCATCAAAAG
2461	ACTTATGATC	CTCTCTCATT	GATTTTTCGC	GGGATACATC	ATCTATTATG	ACGTCAGCCA

Fig. 3 (cont'd)

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2521 TAGCATCAGC ATCCGGCTTA TCCGCCTCCG TTGTCATAAA CCAACGAGGA GGAATATCGT
2581 CGGAGCTGTA CACCATAGCA CTACGTTGAA GATCGTACAG AGCTTTATTA ACTTCTCGCT
2641 TCTCCATATT AAGTTGTCTA GTTAGTTGTG CAGCAGTAGC TCCTTCGATT CCAATGTTTT
2701 TAATAGCCGC ACACACAATC TCTGCGTCAG AACGCTCGTC AATATAGATC TTAGACATTT
2761 TTAGAGAGAA CTAACACAAC CAGCAATAAA ACTGAACCTA CTTTATCATT TTTTTATTCA
2821 TCATCCTCTG GTGGTTCGTC GTTTCTATCG AATGTAGCTC TGATTAACCC GTCATCTATA
2881 GGTGATGCTG GTTCTGGAGA TTCTGGAGGA GATGGATTAT TATCTGGAAG AATCTCTGTT
2941 ATTTCCTTGT TTTCATGTAT CGATTGCGTT GTAACATTAA GATTGCGAAA TGCTCTAAAT
3001 TTGGGAGGCT TAAAGTGTTG TTTGCAATCT CTACACGCGT GTCTAACTAG TGGAGGTTCG
3061 TCAGCTGCTC TAGTTTGAAT CATCATCGGC GTAGTATTCC TACTTTTACA GTTAGGACAC
3121 GGTGTATTGT ATTTCTCGTC GAGAACGTTA AAATAATCGT TGTAACTCAC ATCCTTTATT
3181 TTATCTATAT TGTATTCTAC TCCTTTCTTA ATGCATTTTA TACCGAATAA GAGATAGCGA
3241 AGGAATTCTT TTTATTGATT AACTAGTCAA ATGAGTATAT ATAATTGAAA AAGTAAAATA
3301 TAAATCATAT AATAATGAAA CGAAATATCA GTAATAGACA GGAACTGGCA GATTCTTCTT
3361 CTAATGAAGT AAGTACTGCT AAATCTCCAA AATTAGATAA AAATGATACA GCAAATACAG
3421 CTTCATTCAA CGAATTACCT TTTAATTTTT TCAGACACAC CTTATTACAA ACTAACTAAG
3481 TCAGATGATG AGAAAGTAAA TATAAATTTA ACTTATGGGT ATAATATAAT AAAGATTCAT
3541 GATATTAATA ATTTACTTAA CGATGTTAAT AGACTTATTC CATCAACCCC TTCAAACCTT
3601 TCTGGATATT ATAAAATACC AGTTAATGAT ATTAAAATAG ATTGTTTAAG AGATGTAAAT
3661 AATTATTTGG AGGTAAAGGA TATAAAATTA GTCTATCTTT CACATGGAAA TGAATTACCT
3721 AATATTAATA ATTATGATAG GAATTTTTTA GGATTTACAG CTGTTATATG TATCAACAAT
3781 ACAGGCAGAT CTATGGTTAT GGTAAAACAC TGTAACGGGA AGCAGCATTC TATGGTAACT
3841 GGCCTATGTT TAATAGCCAG ATCATTTTAC TCTATAAACA TTTTACCACA AATAATAGGA
3901 TCCTCTAGAT ATTTAATATT ATATCTAACA ACAACAAAAA AATTTAACGA TGTATGGCCA
3961 GAAGTATTTT CTACTAATAA AGATAAAGAT AGTCTATCTT ATCTACAAGA TATGAAAGAA
4021 GATAATCATT TAGTAGTAGC TACTAATATG GAAAGAAATG TATACAAAAA CGTGGAAGCT
4081 TTTATATTAA ATAGCATATT ACTAGAAGAT TTAAAATCTA GACTTAGTAT AACAAAACAG
4141 TTAAATGCCA ATATCGATTC TATATTTCAT CATAACAGTA GTACATTAAT CAGTGATATA
4201 CTGAAACGAT CTACAGACTC AACTATGCAA GGAATAAGCA ATATGCCAAT TATGTCTAAT
4261 ATTTTAACTT TAGAACTAAA ACGTTCTACC AATACTAAAA ATAGGATACG TGATAGGCTG
4321 TTAAAAGCTG CAATAAATAG TAAGGATGTA GAAGAAATAC TTTGTTCTAT ACCTTCGGAG
4381 GAAAGAACTT TAGAACAACT TAAGTTTAAT CAAACTTGTA TTTATGAAGG TACC
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Fig. 4

Figure 4 DNA sequence of the coding region of FHV gB with modified T5NT motifs.

1	ATGTCCACTC	GTGGCGATCT	TGGGAAGCGG	CGACGAGGGA	GTCGTTGGCA	GGGACACAGT
61	GGCTATTTTC	GACAGAGATG	TTTTTTCCCT	TCTCTACTCG	GTATTGCAGC	GACTGGCTCC
121	AGACATGGTA	ACGGATCGTC	GGGATTAACC	AGACTAGCTA	GATATGTTTC	ATTTATCTGG
181	ATCGTACTAT	TCTTAGTCGG	TCCCCGTCCA	GTAGAGGGTC	AATCTGGAAG	CACATCGGAA
241	CAACCCCGGC	GGACTGTAGC	TACCCCTGAG	GTAGGGGGTA	CACCACCAAA	ACCAACTACA
301	GATCCCACCG	ATATGTCGGA	TATGAGGGAA	GCTCTCCGTG	CGTCCCAAAT	AGAGGCTAAC
361	GGACCATCGA	CTTTCTATAT	GTGTCCACCA	CCTTCAGGAT	CTACTGTCGT	GCGTTTAGAG
421	CCACCACGGG	CCTGTCCAGA	TTATAAACTA	GGGAAAAATT	TTACCGAGGG	TATAGCTGTA
481	ATATTTAAAG	AAAATATAGC	GCCATATAAA	TTCAAGGCAA	ATATATACTA	TAAAAACATT
541	ATTATGACAA	CGGTATGGTC	TGGGAGTTCC	TATGCCGTTA	CAACCAACCG	ATATACAGAC
601	AGGGTTCCCG	TGAAAGTTCA	AGAGATTACA	GATCTCATAG	ATAGACGGGG	TATGTGCCTC
661	TCGAAAGCTG	ATTACGTTCG	TAACAATTAT	CAATTTACGG	CCTTTGATCG	AGACGAGGAT
721	CCCAGAGAAC	TGCCTCTGAA	ACCCTCCAAG	TTCAACACTC	CAGAGTCCCG	TGGATGGCAC
781	ACCACCAATG	AAACATACAC	AAAGATCGGT	GCTGCTGGAT	TTCACCACTC	TGGGACCTCT
841	GTAAATTGCA	TCGTAGAGGA	AGTGGATGCA	AGATCTGTAT	ATCCATATGA	CTCATTTGCT
901	ATCTCCACTG	GTGACGTGAT	TCACATGTCT	CCATTCTTTG	GGCTGAGGGA	TGGAGCCCAT
961	GTAGAACATA	CTAGTTATTC	TTCAGACAGA	TTTCAACAAA	TCGAGGGATA	CTATCCAATA
1021	GACTTGGATA	CGCGATTACA	ACTGGGGGCA	CCAGTTTCTC	GCAATTTTTT	GGAAACTCCG
1081	CATGTGACAG	TGGCCTGGAA	CTGGACCCCA	AAGTCTGGTC	GGGTATGTAC	CTTAGCCAAA
1141	TGGAGGGAAA	TAGATGAAAT	GCTACGCGAT	GAATATCAGG	GCTCCTATAG	ATTTACAGCC
1201	AAGACCATAT	CCGCTACTTT	CATCTCCAAT	ACTTCACAAT	TTGAAATCAA	TCGTATCCGT
1261	TTGGGGGACT	GTGCCACCAA	GGAGGCAGCC	GAAGCCATAG	ACCGGATTTA	TAAGAGTAAA
1321	TATAGTAAAA	CTCATATTCA	GACTGGAACC	CTGGAGACCT	ACCTAGCCCG	TGGGGGATTT
1381	CTAATAGCTT	TCCGTCCCAT	GATCAGCAAC	GAACTAGCAA	AGTTATATAT	CAATGAATTA
1441	GCACGTTCCA	ATCGCACGGT	AGATCTCAGT	GCACTCCTCA	ATCCATCTGG	GGAAACAGTA
1501	CAACGAACTA	GAAGATCGGT	CCCATCTAAT	CAACATCATA	GGTCGCGGCG	CAGCACAATA
1561	GAGGGGGGTA	TAGAAACCGT	GAACAATGCA	TCACTCCTCA	AGACCACCTC	ATCTGTGGAA
1621	TTCGCAATGC	TACAATTTGC	CTATGACTAC	ATACAAGCCC	ATGTAAATGA	AATGTTGAGT
1681	CGGATAGCCA	CTGCCTGGTG	TACACTTCAG	AACCGCGAAC	ATGTGCTGTG	GACAGAGACC
1741	CTAAAACTCA	ATCCCGGTGG	GGTGGTCTCG	ATGGCCCTAG	AACGTCGTGT	ATCCGCGCGC
1801	CTACTTGGAG	ATGCCGTCGC	CGTAACACAA	TGTGTTAACA	TTTCTAGCGG	ACATGTCTAT
1861	ATCCAAAATT	CTATGCGGGT	GACGGGTTCA	TCAACGACAT	GTTACAGCCG	CCCTCTTGTT
1921	TCCTTCCGTG	CCCTCAATGA	CTCCGAATAC	ATAGAAGGAC	AACTAGGGGA	AAACAATGAA
1981	CTTCTCGTGG	AACGAAAACT	AATTGAGCCT	TGCACTGTCA	ATAATAAGCG	GTATTTTAAG
2041	TTTGGGGCAG	ATTATGTATA	TTTTGAGGAT	TATGCGTATG	TCCGTAAAGT	CCCGCTATCG
2101	GAGATAGAAC	TGATAAGTGC	GTATGTGAAT	TTAAATCTTA	CTCTCCTAGA	GGATCGTGAA
2161	TTTCTCCCAC	TCGAAGTTTA	TACACGAGCT	GAGCTGGAAG	ATACCGGCCT	TTTGGACTAC
2221	AGCGAGATTC	AACGCCGCAA	CCAACTCCAC	GCCTTAAAAT	TTTATGATAT	AGACAGCATA
2281	GTCAGAGTGG	ATAATAATCT	TGTCATCATG	CGTGGTATGG	CAAATTTCTT	TCAGGGACTC
2341	GGGGATGTGG	GGGCTGGTTT	CGGCAAGGTG	GTCTTAGGGG	CTGCGAGTGC	GGTAATCTCA
2401	ACAGTATCAG	GCGTATCATC	ATTTCTAAAC	AACCCATTTG	GAGCATTGGC	CGTGGGACTG
2461	TTAATATTAG	CTGGCATCGT	CGCAGCATTC	CTGGCATATC	GCTATATATC	TAGATTACGT
2521	GCAAATCCAA	TGAAAGCCTT	ATATCCTGTG	ACGACTAGGA	ATTTGAAACA	GACGCTAAGA
2581	GCCCGCTCAA	CGGCTGGTGG	GGATAGCGAC	CCGGGAGTCG	ATGACTTCGA	TGAGGAAAAG
2641	CTAATGCAGG	CAAGGGAGAT	GATAAAATAT	ATGTCCCTCG	TATCGGCTAT	GGAGCAACAA
2701	GAACATAAGG	CGATGAAAAA	GAATAAGGGC	CCAGCGATCC	TAACGAGTCA	TCTCACTAAC
2761	ATGGCCCTCC	GTCGCCGTGG	ACCTAAATAC	CAACGCCTCA	ATAATCTTGA	TAGCGGTGAT

Fig. 4 (cont'd)

2821 GATACTGAAA CAAATCTTGT CTAA

Fig. 5

Figure 5 DNA sequence of the the H6 promoted FHV gB donor plasmid pC3H6FHVB.

H6 promoter: 3958 - 3835

FHV gB coding region: 3834 - 991

C3 left arm: 15 - 939

C3 right arm: 4056 - 6628

1	GCGGCCGCGT	CGACATGCAT	TGTTAGTTCT	GTAGATCAGT	AACGTATAGC	ATACGAGTAT
61	AATTATCGTA	GGTAGTAGGT	ATCCTAAAAT	AAATCTGATA	CAGATAATAA	CTTTGTAAAT
121	CAATTCAGCA	ATTTCTCTAT	TATCATGATA	ATGATTAATA	CACAGCGTGT	CGTTATTTTT
181	TGTTACGATA	GTATTTCTAA	AGTAAAGAGC	AGGAATCCCT	AGTATAATAG	AAATAATCCA
241	TATGAAAAAT	ATAGTAATGT	ACATATTTCT	AATGTTAACA	TATTTATAGG	TAAATCCAGG
301	AAGGGTAATT	TTTACATATC	TATATACGCT	TATTACAGTT	ATTAAAAATA	TACTTGCAAA
361	CATGTTAGAA	GTAAAAAAGA	AAGAACTAAT	TTTACAAAGT	GCTTTACCAA	AATGCCAATG
421	GAAATTACTT	AGTATGTATA	TAATGTATAA	AGGTATGAAT	ATCACAAACA	GCAAATCGGC
481	TATTCCCAAG	TTGAGAAACG	GTATAATAGA	TATATTTCTA	GATACCATTA	ATAACCTTAT
541	AAGCTTGACG	TTTCCTATAA	TGCCTACTAA	GAAAACTAGA	AGATACATAC	ATACTAACGC
601	CATACGAGAG	TAACTACTCA	TCGTATAACT	ACTGTTGCTA	ACAGTGACAC	TGATGTTATA
661	ACTCATCTTT	GATGTGGTAT	AAATGTATAA	TAACTATATT	ACACTGGTAT	TTTATTTCAG
721	TTATATACTA	TATAGTATTA	AAAATTATAT	TTGTATAATT	ATATTATTAT	ATTCAGTGTA
781	GAAAGTAAAA	TACTATAAAT	ATGTATCTCT	TATTTATAAC	TTATTAGTAA	AGTATGTACT
841	ATTCAGTTAT	ATTGTTTTAT	AAAAGCTAAA	TGCTACTAGA	TTGATATAAA	TGAATATGTA
901	ATAAATTAGT	AATGTAGTAT	ACTAATATTA	ACTCACATTT	GACTAATTAG	CTATAAAAAC
961	CCGGGCTGCA	GCCCGGGAAG	CTTACAAAAA	TTAGACAAGA	TTTGTTTCAG	TATCATCACC
1021	GCTATCAAGA	TTATTGAGGC	GTTGGTATTT	AGGTCCACGG	CGACGGAGGG	CCATGTTAGT
1081	GAGATGACTC	GTTAGGATCG	CTGGGCCCTT	ATTCTTTTTC	ATCGCCTTAT	GTTCTTGTTG
1141	CTCCATAGCC	GATACGAGGG	ACATATATTT	TATCATCTCC	CTTGCCTGCA	TTAGCTTTTC
1201	CTCATCGAAG	TCATCGACTC	CCGGGTCGCT	ATCCCCACCA	GCCGTTGAGC	GGGCTCTTAG
1261	CGTCTGTTTC	AAATTCCTAG	TCGTCACAGG	ATATAAGGCT	TTCATTGGAT	TTGCACGTAA
1321	TCTAGATATA	TAGCGATATG	CCAGGAATGC	TGCGACGATG	CCAGCTAATA	TTAACAGTCC
1381	CACGGCCAAT	GCTCCAAATG	GGTTGTTTAG	AAATGATGAT	ACGCCTGATA	CTGTTGAGAT
1441	TACCGCACTC	GCAGCCCCTA	AGACCACCTT	GCCGAAACCA	GCCCCCACAT	CCCCGAGTCC
1501	CTGAAAGAAA	TTTGCCATAC	CACGCATGAT	GACAAGATTA	TTATCCACTC	TGACTATGCT
1561	GTCTATATCA	TAAAATTTTA	AGGCGTGGAG	TTGGTTGCGG	CGTTGAATCT	CGCTGTAGTC
1621	CAAAAGGCCG	GTATCTTCCA	GCTCAGCTCG	TGTATAAACT	TCGAGTGGGA	GAAATTCACG
1681	ATCCTCTAGG	AGAGTAAGAT	TTAAATTCAC	ATACGCACTT	ATCAGTTCTA	TCTCCGATAG
1741	CGGGACTTTA	CGGACATACG	CATAATCCTC	AAAATATACA	TAATCTGCCC	CAAACTTAAA
1801	ATACCGCTTA	TTATTGACAG	TGCAAGGCTC	AATTAGTTTT	CGTTCCACGA	GAAGTTCATT
1861	GTTTTCCCCT	AGTTGTCCTT	CTATGTATTC	GGAGTCATTG	AGGGCACGGA	AGGAAACAAG
1921	AGGGCGGCTG	TAACATGTCG	TTGATGAACC	CGTCACCCGC	ATAGAATTTT	GGATATAGAC
1981	ATGTCCGCTA	GAAATGTTAA	CACATTGTGT	TACGGCGACG	GCATCTCCAA	GTAGGCGCGC
2041	GGATACACGA	CGTTCTAGGG	CCATCGAGAC	CACCCCACCG	GGATTGAGTT	TTAGGGTCTC
2101	TGTCCACAGC	ACATGTTCGC	GGTTCTGAAG	TGTACACCAG	GCAGTGGCTA	TCCGACTCAA
2161	CATTTCATTT	ACATGGCTTT	GTATGTAGTC	ATAGGCAAAT	TGTAGCATTG	CGAATTCCAC
2221	AGATGAGGTG	GTCTTGAGGA	GTGATGCATT	GTTCACGGTT	TCTATACCCC	CCTCTATTGT

Fig. 5 (cont')

2281 GCTGCGCCGC GACCTATGAT GTTGATTAGA TGGGACCGAT CTTCTAGTTC GTTGTACTGT
2341 TTCCCCAGAT GGATTGAGGA GTGCACTGAG ATCTACCGTG CGATTGGAAC GTGCTAATTC
2401 ATTGATATAT AACTTTGCTA GTTCGTTGCT GATCATGGGA CGGAAAGCTA TTAGAAATCC
2461 CCCACGGGCT AGGTAGGTCT CCAGGGTTCC AGTCTGAATA TGAGTTTTAC TATATTTACT
2521 CTTATAAATC CGGTCTATGG CTTCGGCTGC CTCCTTGGTG GCACAGTCCC CCAAACGGAT
2581 ACGATTGATT TCAAATTGTG AAGTATTGGA GATGAAAGTA GCGGATATGG TCTTGGCTGT
2641 AAATCTATAG GAGCCCTGAT ATTCATCGCG TAGCATTTCA TCTATTTCCC TCCATTTGGC
2701 TAAGGTACAT ACCCGACCAG ACTTTGGGGT CCAGTTCCAG GCCACTGTCA CATGCGGAGT
2761 TTCCAAAAAA TTGCGAGAAA CTGGTGCCCC CAGTTGTAAT CGCGTATCCA AGTCTATTGG
2821 ATAGTATCCC TCGATTTGTT GAAATCTGTC TGAAGAATAA CTAGTATGTT CTACATGGGC
2881 TCCATCCCTC AGCCCAAAGA ATGGAGACAT GTGAATCACG TCACCAGTGG AGATAGCAAA
2941 TGAGTCATAT GGATATACAG ATCTTGCATC CACTTCCTCT ACGATGCAAT TTACAGAGGT
3001 CCCAGAGTGG TGAAATCCAG CAGCACCGAT CTTTGTGTAT GTTTCATTGG TGGTGTGCCA
3061 TCCACGGGAC TCTGGAGTGT TGAACTTGGA GGGTTTCAGA GGCAGTTCTC TGGGATCCTC
3121 GTCTCGATCA AAGGCCGTAA ATTGATAATT GTTACGAACG TAATCAGCTT TCGAGAGGCA
3181 CATACCCCGT CTATCTATGA GATCTGTAAT CTCTTGAACT TTCACGGGAA CCCTGTCTGT
3241 ATATCGGTTG GTTGTAACGG CATAGGAACT CCCAGACCAT ACCGTTGTCA TAATAATGTT
3301 TTTATAGTAT ATATTTGCCT TGAATTTATA TGGCGCTATA TTTTCTTTAA ATATTACAGC
3361 TATACCCTCG GTAAAATTTT TCCCTAGTTT ATAATCTGGA CAGGCCCGTG GTGGCTCTAA
3421 ACGCACGACA GTAGATCCTG AAGGTGGTGG ACACATATAG AAAGTCGATG GTCCGTTAGC
3481 CTCTATTTGG GACGCACGGA GAGCTTCCCT CATATCCGAC ATATCGGTGG GATCTGTAGT
3541 TGGTTTTGGT GGTGTACCCC CTACCTCAGG GGTAGCTACA GTCCGCCGGG GTTGTTCCGA
3601 TGTGCTTCCA GATTGACCCT CTACTGGACG GGGACCGACT AAGAATAGTA CGATCCAGAT
3661 AAATGAAACA TATCTAGCTA GTCTGGTTAA TCCCGACGAT CCGTTACCAT GTCTGGAGCC
3721 AGTCGCTGCA ATACCGAGTA GAGAAGGGAA AAAACATCTC TGTCGAAAAT AGCCACTGTG
3781 TCCCTGCCAA CGACTCCCTC GTCGCCGCTT CCCAAGATCG CCACGAGTGG ACATTACGAT
3841 ACAAACTTAA CGGATATCGC GATAATGAAA TAATTTATGA TTATTTCTCG CTTTCAATTT
3901 AACACAACCC TCAAGAACCT TTGTATTTAT TTTCACTTTT TAAGTATAGA ATAAAGAAGC
3961 TCTAATTAAT TAAGCTACAA ATAGTTTCGT TTTCACCTTG TCTAATAACT AATTAATTAA
4021 CCCGGATCGA TCCCGATTTT TATGACTAGT TAATCAAATA AAAAGCATAC AAGCTATTGC
4081 TTCGCTATCG TTACAAAATG GCAGGAATTT TGTGTAAACT AAGCCACATA CTTGCCAATG
4141 AAAAAAATAG TAGAAAGGAT ACTATTTTAA TGGGATTAGA TGTTAAGGTT CCTTGGGATT
4201 ATAGTAACTG GGCATCTGTT AACTTTTACG ACGTTAGGTT AGATACTGAT GTTACAGATT
4261 ATAATAATGT TACAATAAAA TACATGACAG GATGTGATAT TTTTCCTCAT ATAACTCTTG
4321 GAATAGCAAA TATGGATCAA TGTGATAGAT TTGAAAATTT CAAAAAGCAA ATAACTGATC
4381 AAGATTTACA GACTATTTCT ATAGTCTGTA AAGAAGAGAT GTGTTTTCCT CAGAGTAACG
4441 CCTCTAAACA GTTGGGAGCG AAAGGATGCG CTGTAGTTAT GAAACTGGAG GTATCTGATG
4501 AACTTAGAGC CCTAAGAAAT GTTCTGCTGA ATGCGGTACC CTGTTCGAAG GACGTGTTTG
4561 GTGATATCAC AGTAGATAAT CCGTGGAATC CTCACATAAC AGTAGGATAT GTTAAGGAGG
4621 ACGATGTCGA AAACAAGAAA CGCCTAATGG AGTGCATGTC CAAGTTTAGG GGGCAAGAAA
4681 TACAAGTTCT AGGATGGTAT TAATAAGTAT CTAAGTATTT GGTATAATTT ATTAAATAGT
4741 ATAATTATAA CAAATAATAA ATAACATGAT AACGGTTTTT ATTAGAATAA AATAGAGATA
4801 ATATCATAAT GATATATAAT ACTTCATTAC CAGAAATGAG TAATGGAAGA CTTATAAATG
4861 AACTGCATAA AGCTATAAGG TATAGAGATA TAAATTTAGT AAGGTATATA CTTAAAAAAT
4921 GCAAATACAA TAACGTAAAT ATACTATCAA CGTCTTTGTA TTTAGCCGTA AGTATTTCTG
4981 ATATAGAAAT GGTAAAATTA TTACTAGAAC ACGGTGCCGA TATTTTAAAA TGTAAAAATC
5041 CTCCTCTTCA TAAAGCTGCT AGTTTAGATA ATACAGAAAT TGCTAAACTA CTAATAGATT
5101 CTGGCGCTGA CATAGAACAG ATACATTCTG GAAATAGTCC GTTATATATT TCTGTATATA
5161 GAAACAATAA GTCATTAACT AGATATTTAT TAAAAAAAGG TGTTAATTGT AATAGATTCT
5221 TTCTAAATTA TTACGATGTA CTGTATGATA AGATATCTGA TGATATGTAT AAAATATTTA
5281 TAGATTTTAA TATTGATCTT AATATACAAA CTAGAAATTT TGAAACTCCG TTACATTACG
5341 CTATAAAGTA TAAGAATATA GATTTAATTA GGATATTGTT AGATAATAGT ATTAAAATAG
5401 ATAAAAGTTT ATTTTTGCAT AAACAGTATC TCATAAAGGC ACTTAAAAAT AATTGTAGTT
5461 ACGATATAAT AGCGTTACTT ATAAATCACG GAGTGCCTAT AAACGAACAA GATGATTTAG

Fig. 5 (cont'd)

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5521 GTAAAACCCC ATTACATCAT TCGGTAATTA ATAGAAGAAA AGATGTAACA GCACTTCTGT
5581 TAAATCTAGG AGCTGATATA AACGTAATAG ATGACTGTAT GGGCAGTCCC TTACATTACG
5641 CTGTTTCACG TAACGATATC GAAACAACAA AGACACTTTT AGAAAGAGGA TCTAATGTTA
5701 ATGTGGTTAA TAATCATATA GATACCGTTC TAAATATAGC TGTTGCATCT AAAAACAAAA
5761 CTATAGTAAA CTTATTACTG AAGTACGGTA CTGATACAAA GTTGGTAGGA TTAGATAAAC
5821 ATGTTATTCA CATAGCTATA GAAATGAAAG ATATTAATAT ACTGAATGCG ATCTTATTAT
5881 ATGGTTGCTA TGTAAACGTC TATAATCATA AAGGTTTCAC TCCTCTATAC ATGGCAGTTA
5941 GTTCTATGAA AACAGAATTT GTTAAACTCT TACTTGACCA CGGTGCTTAC GTAAATGCTA
6001 AAGCTAAGTT ATCTGGAAAT ACTCCTTTAC ATAAAGCTAT GTTATCTAAT AGTTTTAATA
6061 ATATAAAATT ACTTTTATCT TATAACGCCG ACTATAATTC TCTAAATAAT CACGGTAATA
6121 CGCCTCTAAC TTGTGTTAGC TTTTTAGATG ACAAGATAGC TATTATGATA ATATCTAAAA
6181 TGATGTTAGA AATATCTAAA AATCCTGAAA TAGCTAATTC AGAAGGTTTT ATAGTAAACA
6241 TGGAACATAT AAACAGTAAT AAAAGACTAC TATCTATAAA AGAATCATGC GAAAAAGAAC
6301 TAGATGTTAT AACACATATA AAGTTAAATT CTATATATTC TTTTAATATC TTTCTTGACA
6361 ATAACATAGA TCTTATGGTA AAGTTCGTAA CTAATCCTAG AGTTAATAAG ATACCTGCAT
6421 GTATACGTAT ATATAGGGAA TTAATACGGA AAAATAAATC ATTAGCTTTT CATAGACATC
6481 AGCTAATAGT TAAAGCTGTA AAAGAGAGTA AGAATCTAGG AATAATAGGT AGGTTACCTA
6541 TAGATATCAA ACATATAATA ATGGAACTAT TAAGTAATAA TGATTTACAT TCTGTTATCA
6601 CCAGCTGTTG TAACCCAGTA GTATAAAG
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Fig. 6

POL/NEF epitopes

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        10         20         30         40         50         60         70         80         90        100        110
TTTTTTTCAT TATTTAGAAA TTATGCATTT TAGATCTTTA TAAGCGGCCG TGATTAACTA GTCATAAAAA CCCGGGATCG ATTCTAGACT CGAGGGTACC GGATCTTAAT
AAAAAAAGTA ATAAATCTTT AATACGTAAA ATCTAGAAAT ATTCGCCGGC ACTAATTGAT CAGTATTTTT GGGCCCTAGC TAAGATCTGA GCTCCCATGG CCTAGAATTA

       120        130        140        150        160        170        180        190        200        210        220
TAATTAGTCA TCAGGCAGGG CGAGAACGAG ACTATCTGCT CGTTAATTAA TTAGGTCGAC GGATCCCCCA ACAAAAACTA ATCAGCTATC GGGGTTAATT AATTAGTTAT
ATTAATCAGT AGTCCGTCCC GCTCTTGCTC TGATAGACGA GCAATTAATT AATCCAGCTG CCTAGGGGGT TGTTTTTGAT TAGTCGATAG CCCCAATTAA TTAATCAATA

       230        240        250        260        270        280        290        300        310        320        330
TAGACAAGGT GAAAACGAAA CTATTTGTAG CTTAATTAAT TAGAGCTTCT TTATTCTATA CTTAAAAAGT GAAAATAAAT ACAAAGGTTC TTGAGGGTTG TGTTAAATTG
ATCTGTTCCA CTTTTGCTTT GATAAACATC GAATTAATTA ATCTCGAAGA AATAAGATAT GAATTTTTCA CTTTTATTTA TGTTTCCAAG AACTCCCAAC ACAATTTAAC
                                                 ____________________________H6 promoter_____________________________>

       340        350        360        370        380        390          400          410          420          430
AAAGCGAGAA ATAATCATAA ATTATTTCAT TATCGCGATA TCCGTTAAGT TTGTATCGTA ATG CCA CTA ACA GAA GAA GCA GAG CTA GAA CTG GCA GAA AAC
TTTCGCTCTT TATTAGTATT TAATAAAGTA ATAGCGCTAT AGGCAATTCA AACATAGCAT TAC GGT GAT TGT CTT CTT CGT CTC GAT CTT GAC CGT CTT TTG
___________________________H6 promoter_________________________> Met Pro Leu Thr Glu Glu Ala Glu Leu Glu Leu Ala Glu Asn
                                                                  ____________________POL/NEF Epitopes_________________>

       440         450          460          470          480          490          500          510          520
AGA GAG ATT CTA AAA GAA CCA GTA CAT GGA GTG TAT TAT GAC CCA TCA AAA GAC TTA ATA GCA GAA ATA CAG AAG CAG GGG CAA GGC CAA
TCT CTC TAA GAT TTT CTT GGT CAT GTA CCT CAC ATA ATA CTG GGT AGT TTT CTG AAT TAT CGT CTT TAT GTC TTC GTC CCC GTT CCG GTT
Arg Glu Ile Leu Lys Glu Pro Val His Gly Val Tyr Tyr Asp Pro Ser Lys Asp Leu Ile Ala Glu Ile Gln Lys Gln Gly Gln Gly Gln
______________________________________________POL/NEF Epitopes_______________________________________________________>

       530         540          550          560          570          580          590          600          610
TGG ACA TAT CAA ATT TAT CAA GAG CCA TTT AAA AAT CTG AAA ACA GGA ATG GAG TGG AGA TTT GAT TCT AGA TTA GCA TTT CAT CAC GTA
ACC TGT ATA GTT TAA ATA GTT CTC GGT AAA TTT TTA GAC TTT TGT CCT TAC CTC ACC TCT AAA CTA AGA TCT AAT CGT AAA GTA GTG CAT
Trp Thr Tyr Gln Ile Tyr Gln Glu Pro Phe Lys Asn Leu Lys Thr Gly Met Glu Trp Arg Phe Asp Ser Arg Leu Ala Phe His His Val
______________________________________________POL/NEF Epitopes_______________________________________________________>

       620         630          640          650          660          670          680          690          700
GCT AGA GAA TTA CAT CCT GAA TAT TTT AAA AAT TGT AAG CTT ATG GCA ATA TTC CAA AGT AGC ATG ACA AAA ATC TTA GAG CCT TTT AGA
CGA TCT CTT AAT GTA GGA CTT ATA AAA TTT TTA ACA TTC GAA TAC CGT TAT AAG GTT TCA TCG TAC TGT TTT TAG AAT CTC GGA AAA TCT
Ala Arg Glu Leu His Pro Glu Tyr Phe Lys Asn Cys Lys Leu Met Ala Ile Phe Gln Ser Ser Met Thr Lys Ile Leu Glu Pro Phe Arg
______________________________________________POL/NEF Epitopes_______________________________________________________>

       710         720          730          740          750          760          770          780          790
AAA CAA AAT CCA GAC ATA GTT ATC TAT CAA TAC ATG GAT GAT TTG TAT GTA GGA TCT GAC TTA GAA ATA GGG CAG CAT AGA ACA AAA ATA
TTT GTT TTA GGT CTG TAT CAA TAG ATA GTT ATG TAC CTA CTA AAC ATA CAT CCT AGA CTG AAT CTT TAT CCC GTC GTA TCT TGT TTT TAT
Lys Gln Asn Pro Asp Ile Val Ile Tyr Gln Tyr Met Asp Asp Leu Tyr Val Gly Ser Asp Leu Glu Ile Gly Gln His Arg Thr Lys Ile
______________________________________________POL/NEF Epitopes_______________________________________________________>

       800         810          820          830          840          850          860          870          880
GAG GAG CTG AGA CAA CAT CTG TTG AGG TGG GGA CTT ACA ACC ATG GTA GGT TTT CCA GTA ACA CCT CAA GTA CCT TTA AGA CCA ATG ACT
CTC CTC GAC TCT GTT GTA GAC AAC TCC ACC CCT GAA TGT TGG TAC CAT CCA AAA GGT CAT TGT GGA GTT CAT GGA AAT TCT GGT TAC TGA
Glu Glu Leu Arg Gln His Leu Leu Arg Trp Gly Leu Thr Thr Met Val Gly Phe Pro Val Thr Pro Gln Val Pro Leu Arg Pro Met Thr
______________________________________________POL/NEF Epitopes_______________________________________________________>
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Fig. 6 (cont'd)

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     890       900       910       920       930       940       950       960       970
TAC AAA GCA GCT GTA GAT CTT TCT CAC TTT TTA AAA GAA AAA GGA GGT TTA GAA GGG CTA ATT CAT TCT CAA CGA AGA CAA GAT ATT CTT
ATG TTT CGT CGA CAT CTA GAA AGA GTG AAA AAT TTT CTT TTT CCT CCA AAT CTT CCC GAT TAA GTA AGA GTT GCT TCT GTT CTA TAA GAA
Tyr Lys Ala Ala Val Asp Leu Ser His Phe Leu Lys Glu Lys Gly Gly Leu Glu Gly Leu Ile His Ser Gln Arg Arg Gln Asp Ile Leu
______________________________________POL/NEF Epitopes______________________________________>

     980       990      1000      1010      1020      1030      1040      1050      1060
GAT TTG TGG ATT TAT CAT ACA CAA GGA TAT TTT CCT GAT TGG CAG AAT TAC ACA CCA GGA CCA GGA GTC AGA TAC CCA TTA ACC TTT GGT
CTA AAC ACC TAA ATA GTA TGT GTT CCT ATA AAA GGA CTA ACC GTC TTA ATG TGT GGT CCT GGT CCT CAG TCT ATG GGT AAT TGG AAA CCA
Asp Leu Trp Ile Tyr His Thr Gln Gly Tyr Phe Pro Asp Trp Gln Asn Tyr Thr Pro Gly Pro Gly Val Arg Tyr Pro Leu Thr Phe Gly
______________________________________POL/NEF Epitopes______________________________________>

    1070      1080      1090      1100      1110      1120      1130      1140      1150
TGG TGC TAC AAG CTA GTA CCA ATG ATT GAG ACT GTA CCA GTA AAA TTA AAG CCA GGA ATG GAT GGC CCA AAA GTT AAA CAA TGG CCA TTG
ACC ACG ATG TTC GAT CAT GGT TAC TAA CTC TGA CAT GGT CAT TTT AAT TTC GGT CCT TAC CTA CCG GGT TTT CAA TTT GTT ACC GGT AAC
Trp Cys Tyr Lys Leu Val Pro Met Ile Glu Thr Val Pro Val Lys Leu Lys Pro Gly Met Asp Gly Pro Lys Val Lys Gln Trp Pro Leu
______________________________________POL/NEF Epitopes______________________________________>

    1160      1170      1180      1190      1200      1210      1220      1230      1240
ACA GAA GAA AAA ATA AAA GCA TTA GTA GAA ATT TGT ACA GAG ATG GAA AAG GAA GGG AAA ATT TCA AAA ATT GGG CCT TAA TTTTTCT
TGT CTT CTT TTT TAT TTT CGT AAT CAT CTT TAA ACA TGT CTC TAC CTT TTC CTT CCC TTT TAA AGT TTT TAA CCC GGA ATT AAAAAGA
Thr Glu Glu Lys Ile Lys Ala Leu Val Glu Ile Cys Thr Glu Met Glu Lys Glu Gly Lys Ile Ser Lys Ile Gly Pro
________________________________POL/NEF Epitopes________________________________>

      1250       1260       1270       1280       1290       1300       1310       1320       1330       1340       1350
GCAGCCCGGG GGATCCTTTT TATAGCTAAT TAGTCACGTA CCTTTGAGAG TACCACTTCA GCTACCTCTT TTGTGTCTCA GAGTAACTTT CTTTAATCAA TTCCAAAACA
CGTCGGGCCC CCTAGGAAAA ATATCGATTA ATCAGTGCAT GGAAACTCTC ATGGTGAAGT CGATGGAGAA AACACAGAGT CTCATTGAAA GAAATTAGTT AAGGTTTTGT
```

Fig. 7

gag (+ pro) and gp120 (+ transmembrane)

FEATURES	From	To/Span	Description
frag	1	56	C3 flanking arm
frag	162	76 (C)	HIV1 (IIIB) env transmembrane region
frag	1728	163 (C)	HIV1 (MN) gp120 gene
frag	1853	1729 (C)	vaccinia H6 promoter
frag	1925	1983	vaccinia I3L promoter
frag	1984	3746	HIV1 (IIIB) gag/pro gene
frag	3753	3808	C3 flanking arm

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        10         20         30         40         50         60         70         80         90        100        110        120
TAATGTAGTATACTAATATTAACTCACATTTGACTAATTAGCTATAAAAACCCGGGATCGATTCTAGAATAAAAATTATCCCTGCCTAACTCTATTCACTACAGAGAGTACAGCAAAAAC
ATTACATCATATGATTATAATTGAGTGTAAACTGATTAATCGATATTTTTGGGCCCTAGCTAAGATCTTATTTTTAATAGGGACGGATTGAGATAAGTGATGTCTCTCATGTCGTTTTTG
____________________C3 FLANKING ARM____________________>                                   G  Q  R  V  R  N  V  V  S  L  V  A  F  V
                                                                                     <____HIV1 (IIIB) ENV TRANSMEMBRANE REGION_____

       130        140        150        160        170        180        190        200        210        220        230        240
TATTCTTAAACCTACCAAGCCTCCTACTATCATTATGAATAATCTTTTTTCTCTCTGCACCACTCTTCTCTTTGCCTTGGTGGGTGCTACTCCTAATGGTTCAATTGTTACTACTTTATA
ATAAGAATTTGGATGGTTCGGAGGATGATAGTAATACTTATTAGAAAAAAGAGAGACGTGGTGAGAAGAGAAACGGAACCACCCACGATGAGGATTACCAAGTTAACAATGATGAAATAT
 I  R  L  G  V  L  G  G  V  I  M  I  F  L  R  K  E  R  Q  V  V  R  R  K  A  K  T  P  A  V  G  L  P  E  I  T  V  V  K  Y
<__HIV1 (IIIB) ENV TRANSMEMBRANE REGION___
                                          <___________________________HIV1 (MN) GP120 GENE______________________________

       250        260        270        280        290        300        310        320        330        340        350        360
TTTATATAATTCACTTCTCCAATTGTCCCTCATATCTCCTCCTCCAGGTCTGAAGATCTCGGTGTCGTTCGTGTCCGTGTCCTTACCACCATCTCTTGTTAATAGTAGCCCTGTAATATT
AAATATATTAAGTGAAGAGGTTAACAGGGAGTATAGAGGAGGAGGTCCAGACTTCTAGAGCCACAGCAAGCACAGGCACAGGAATGGTGGTAGAGAACAATTATCATCGGGACATTATAA
 K  Y  L  E  S  R  W  N  D  R  M  D  G  G  G  P  R  F  I  E  T  D  N  T  D  T  D  K  G  G  D  R  T  L  L  L  G  T  I  N
<_____________________________________________HIV1 (MN) GP120 GENE______________________________________________

       370        380        390        400        410        420        430        440        450        460        470        480
TGATGAACATCTAATTTGTCCTTCAATGGGAGGGGCATATATTGCTTTTCCTACTTCCTGCCACATGTTTATAATTTGTTTTATTTTGCATTGAAGTGTGATATTGTTATTTGACCCTGT
ACTACTTGTAGATTAAACAGGAAGTTACCCTCCCCGTATATAACGAAAAGGATGAAGGACGGTGTACAAATATTAAACAAAATAAAACGTAACTTCACACTATAACAATAAACTGGGACA
 S  S  C  R  I  Q  G  E  I  P  P  A  Y  I  A  K  G  V  E  Q  W  M  N  I  I  Q  K  I  K  C  Q  L  T  I  N  N  N  S  G  T
<_____________________________________________HIV1 (MN) GP120 GENE______________________________________________

       490        500        510        520        530        540        550        560        570        580        590        600
AGTATTATTCCAAGTATTATTACCATTCCAAGTACTATTAAACAGTGGTGATGAATTACAGTAGAAGAATTCCCCTCCACAATTAAAACTGTGCATTACAATTTCTGGGTCCCCTCCTGA
TCATAATAAGGTTCATAATAATGGTAAGGTTCATGATAATTTGTCACCACTACTTAATGTCATCTTCTTAAGGGGAGGTGTTAATTTTGACACGTAATGTTAAAGACCCAGGGGAGGACT
 T  N  N  W  T  N  N  G  N  W  T  S  N  F  L  P  S  S  N  C  Y  F  F  E  G  G  C  N  F  S  H  M  V  I  E  P  D  G  G  S
<_____________________________________________HIV1 (MN) GP120 GENE______________________________________________

       610        620        630        640        650        660        670        680        690        700        710        720
GGATTGATTAAAGACTATTGTTTTATTCTTAAATTGTTCTTTTAATTTGCTAACTATCTGTCTTAAAGTGTCATTCCATTTTGCTCTACTAATGTTACAATGTGCTTGTCTTATAGTTCC
CCTAACTAATTTCTGATAACAAAATAAGAATTTAACAAGAAAATTAAACGATTGATAGACAGAATTTCACAGTAAGGTAAAACGAGATGATTACAATGTTACACGAACAGAATATCAAGG
 S  Q  N  F  V  I  T  K  N  K  F  Q  E  K  L  K  S  V  I  Q  R  L  T  D  N  W  K  A  R  S  I  N  C  H  A  Q  R  I  T  G
<_____________________________________________HIV1 (MN) GP120 GENE______________________________________________

       730        740        750        760        770        780        790        800        810        820        830        840
TATTATATTTTTTGTTGTATAAAATGCTCTCCCTGGTCCTATATGTATCCTTTTTCTTTTATTGTAGTTGGGTCTTGTACAATTAATTTGTACAGATTCATTCAGATGTACTATGATGGT
ATAATATAAAAAACAACATATTTTACGAGAGGGACCAGGATATACATAGGAAAAAGAAAATAACATCAACCCAGAACATGTTAATTAAACATGTCTAAGTAAGTCTACATGATACTACCA
 I  I  N  K  T  T  Y  F  A  R  G  P  G  I  H  I  R  K  R  K  N  Y  N  P  R  T  C  N  I  Q  V  S  E  N  L  H  V  I  I  T
<_____________________________________________HIV1 (MN) GP120 GENE______________________________________________

       850        860        870        880        890        900        910        920        930        940        950        960
TTTAGCATTATCATTGAAATTCTCAGATCTAATTACTACCTCTTCTTCTGCTAGACTGCCATTTAACAGCAGTTGAGTTGATACTACTGGCCTAATTCCATGTGTACATTGTACTGTGCT
AAATCGTAATAGTAACTTTAAGAGTCTAGATTAATGATGGAGAAGAAGACGATCTGACGGTAAATTGTCGTCAACTCAACTATGATGACCGGATTAAGGTACACATGTAACATGACACGA
 K  A  N  D  N  F  N  E  S  R  I  V  V  E  E  E  A  L  S  G  N  L  L  L  Q  T  S  V  V  P  R  I  G  H  T  C  Q  V  T  S
<_____________________________________________HIV1 (MN) GP120 GENE______________________________________________
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Fig. 7 (cont'd)

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      970       980       990      1000      1010      1020      1030      1040      1050      1060      1070      1080
GACATTTTTACATGATCCTTTTCCACTGAACTTTTTATCGTTACACTTTAGAATCGCAAAACCAGCCGGGGCACAATAGTGTATGGGAATTGGCTCAAAGGATATCTTTGGACAAGCTTG
CTGTAAAAATGTACTAGGAAAAGGTGACTTGAAAAATAGCAATGTGAAATCTTAGCGTTTTGGTCGGCCCCGTGTTATCACATACCCTTAACCGAGTTTCCTATAGAAACCTGTTCGAAC
 V  N  K  C  S  G  K  G  S  F  K  K  D  N  C  K  L  I  A  F  G  A  P  A  C  Y  H  I  P  I  P  E  F  S  I  K  P  C  A  Q
<________________________________________________HIV1 (MN) GP120 GENE____________________________________________________

     1090      1100      1110      1120      1130      1140      1150      1160      1170      1180      1190      1200
TGTAATGACTGAGGTATTACAACTTATCAACCTATAGCTGGTACTATCATTATTTATTGATACTATATCAAGTTTATAAAGAAGTGCATATTCTTTCTGCATCTTATCTCTTATGCTTGT
ACATTACTGACTCCATAATGTTGAATAGTTGGATATCGACCATGATAGTAATAAATAACTATGATATAGTTCAAATATTTCTTCACGTATAAGAAAGACGTAGAATAGAGAATACGAACA
 T  I  V  S  T  N  C  S  I  L  R  Y  S  T  S  D  N  N  I  S  V  I  D  L  K  Y  L  L  A  Y  E  K  Q  M  K  D  R  I  S  T
<________________________________________________HIV1 (MN) GP120 GENE____________________________________________________

     1210      1220      1230      1240      1250      1260      1270      1280      1290      1300      1310      1320
GGTGATATTGAAAGAGCAGTTTTTCATTTCTCCTCCCTTTATTGTTCCCTCGCTATTACTATTGTTATTAGCAGTACTATTATTGGTATTAGTAGTATTCCTCAAATCAGTGCAATTTAA
CCACTATAACTTTCTCGTCAAAAAGTAAAGAGGAGGGAAATAACAAGGGAGCGATAATGATAACAATAATCGTCATGATAATAACCATAATCATCATAAGGAGTTTAGTCACGTTAAATT
 T  I  N  F  S  C  N  K  M  E  G  G  K  I  T  G  E  S  N  S  N  N  N  A  T  S  N  N  T  N  T  T  N  R  L  D  T  C  N  L
<________________________________________________HIV1 (MN) GP120 GENE____________________________________________________

     1330      1340      1350      1360      1370      1380      1390      1400      1410      1420      1430      1440
AGTAACACAGAGTGGGGTTAATTTTACACATGGCTTTAGGCTTTGATCCCATAAACTGATTATATCCTCATGCATCTGTTCTACCATGTTATTTTTCCACATGTTAAAATTTTCTGTCAC
TCATTGTGTCTCACCCCAATTAAAATGTGTACCGAAATCCGAAACTAGGGTATTTGACTAATATAGGAGTACGTAGACAAGATGGTACAATAAAAAGGTGTACAATTTTAAAAGACAGTG
 T  V  C  L  P  T  L  K  V  C  P  K  L  S  Q  D  W  L  S  I  I  D  E  H  M  Q  E  V  M  N  N  K  W  M  N  F  N  E  T  V
<________________________________________________HIV1 (MN) GP120 GENE____________________________________________________

     1450      1460      1470      1480      1490      1500      1510      1520      1530      1540      1550      1560
ATTTACCAATTCTACTTCTTGTGGGTTGGGGTCTGTGGGTACACAGGCATGTGTGGCCCAAACATTATGTACCTCTGTATCATATGCTTTAGCATCTGATGCACAAAATAGAGTGGTGGT
TAAATGGTTAAGATGAAGAACACCCAACCCCAGACACCCATGTGTCCGTACACACCGGGTTTGTAATACATGGAGACATAGTATACGAAATCGTAGACTACGTGTTTTATCTCACCACCA
 N  V  L  E  V  E  Q  P  N  P  D  T  P  V  C  A  H  T  A  W  V  N  H  V  E  T  D  Y  A  K  A  D  S  A  C  F  L  T  T  T
<________________________________________________HIV1 (MN) GP120 GENE____________________________________________________

     1570      1580      1590      1600      1610      1620      1630      1640      1650      1660      1670      1680
TGCTTCTTTCCACACAGGTACCCCATAATAGACTGTGACCCACAATTTTTCTGTAGCACTACAGATCATCAACATCCCAAGGAGCATGGTGCCCCATCTCCACCCCCATCTCCACAAGTG
ACGAAGAAAGGTGTGTCCATGGGGTATTATCTGACACTGGGTGTTAAAAAGACATCGTGATGTCTAGTAGTTGTAGGGTTCCTCGTACCACGGGGTAGAGGTGGGGGTAGAGGTGTTCAC
 A  E  K  W  V  P  V  G  Y  Y  V  T  V  W  L  K  E  T  A  S  C  I  M  L  M  G  L  L  M  T  G  W  R  W  G  W  R  W  L  H
<________________________________________________HIV1 (MN) GP120 GENE____________________________________________________

     1690      1700      1710      1720      1730      1740      1750      1760      1770      1780      1790      1800
CTGATATTTCTCCTTCACTCTCATTGCCACTGTCTTCTGCTCTTTCATATACGATACAAACTTAACGCATATCGCGATAATGAAATAATTTATGATTATTTCTCGCTTTCAATTTAACAC
GACTATAAAGAGGAAGTGAGAGTAACGGTGACAGAAGACGAGAAAGTATATGCTATGTTTGAATTGCGTATAGCGCTATTACTTTATTAAATACTAATAAAGAGCGAAAGTTAAATTGTG
 Q  Y  K  E  K  V  R  M  A  V  T  K  Q  E  K  M
<___________HIV1 (MN) GP120 GENE_______________
                                                <________________________VACCINIA H6 PROMOTER_____________________

     1810      1820      1830      1840      1850      1860      1870      1880      1890      1900      1910      1920
AACCCTCAAGAACCTTTGTATTTATTTTCACTTTTTAAGTATAGAATAAAGAAGCTCTAATTAATTAAGCTACAAATAGTTTCGTTTTCACCTTGTCTAATAACTAATTAATTAACCCGG
TTGGGAGTTCTTGGAAACATAAATAAAAGTGAAAAATTCATATCTTATTTCTTCGAGATTAATTAATTCGATGTTTATCAAAGCAAAAGTGGAACAGATTATTGATTAATTAATTGGGCC
<_____________VACCINIA H6 PROMOTER_________________

     1930      1940      1950      1960      1970      1980      1990      2000      2010      2020      2030      2040
ATCTTGAGATAAAGTGAAAATATATATCATTATATTACAAAGTACAATTATTTAGGTTTAATCATGGGTGCGAGAGCGTCAGTATTAAGCGGGGGAGAATTAGATCGATGGGAAAAAATT
TAGAACTCTATTTCACTTTTATATATAGTAATATAATGTTTCATGTTAATAAATCCAAATTAGTACCCACGCTCTCGCAGTCATAATTCGCCCCCTCTTAATCTAGCTACCCTTTTTTAA
   ________________VACCINIA I3L PROMOTER__________________> M  G  A  R  A  S  V  L  S  G  G  E  L  D  R  W  E  K  I
                                                          _______________HIV1 (IIIB) GAG/PRO GENE________________>

     2050      2060      2070      2080      2090      2100      2110      2120      2130      2140      2150      2160
CGGTTAAGGCCAGGGGGAAAGAAAAAATATAAATTAAAACATATAGTATGGGCAAGCAGGGAGCTAGAACGATTCGCAGTTAATCCTGGCCTGTTAGAAACATCAGAAGGCTGTAGACAA
GCCAATTCCGGTCCCCCTTTCTTTTTTATATTTAATTTTGTATATCATACCCGTTCGTCCCTCGATCTTGCTAAGCGTCAATTAGGACCGGACAATCTTTGTAGTCTTCCGACATCTGTT
 R  L  R  P  G  G  K  K  K  Y  K  L  K  H  I  V  W  A  S  R  E  L  E  R  F  A  V  N  P  G  L  L  E  T  S  E  G  C  R  Q
_______________________________________________HIV1 (IIIB) GAG/PRO GENE___________________________________________________>

     2170      2180      2190      2200      2210      2220      2230      2240      2250      2260      2270      2280
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Fig. 7 (cont'd)

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ATACTGGGACAGCTACAACCATCCCTTCAGACAGGATCAGAAGAACTTAGATCATTATATAATACAGTAGCAACCCTCTATTGTGTGCATCAAAGGATAGAGATAAAAGACACCAAGGAA
TATGACCCTGTCGATGTTGGTAGGGAAGTCTGTCCTAGTCTTCTTGAATCTAGTAATATATTATGTCATCGTTGGGAGATAACACACGTAGTTTCCTATCTCTATTTTCTGTGGTTCCTT
 I  L  G  Q  L  Q  P  S  L  Q  T  G  S  E  E  L  R  S  L  Y  N  T  V  A  T  L  Y  C  V  H  Q  R  I  E  I  K  D  T  K  E
__________________________________________HIV1 (IIIB) GAG/PRO GENE_________________________________________>

      2290      2300      2310      2320      2330      2340      2350      2360      2370      2380      2390      2400
GCTTTAGACAAGATAGAGGAAGAGCAAAACAAAAGTAAGAAAAAAGCACAGCAAGCAGCAGCTGACACAGGACACAGCAATCAGGTCAGCCAAAATTACCCTATAGTGCAGAACATCCAG
CGAAATCTGTTCTATCTCCTTCTCGTTTTGTTTTCATTCTTTTTTCGTGTCGTTCGTCGTCGACTGTGTCCTGTGTCGTTAGTCCAGTCGGTTTTAATGGGATATCACGTCTTGTAGGTC
 A  L  D  K  I  E  E  E  Q  N  K  S  K  K  K  A  Q  Q  A  A  A  D  T  G  H  S  N  Q  V  S  Q  N  Y  P  I  V  Q  N  I  Q
__________________________________________HIV1 (IIIB) GAG/PRO GENE_________________________________________>

      2410      2420      2430      2440      2450      2460      2470      2480      2490      2500      2510      2520
GGGCAAATGGTACATCAGGCCATATCACCTAGAACTTTAAATGCATGGGTAAAAGTAGTAGAAGAGAAGGCTTTCAGCCCAGAAGTGATACCCATGTTTTCAGCATTATCAGAAGGAGCC
CCCGTTTACCATGTAGTCCGGTATAGTGGATCTTGAAATTTACGTACCCATTTTCATCATCTTCTCTTCCGAAAGTCGGGTCTTCACTATGGGTACAAAAGTCGTAATAGTCTTCCTCGG
 G  Q  M  V  H  Q  A  I  S  P  R  T  L  N  A  W  V  K  V  V  E  E  K  A  F  S  P  E  V  I  P  M  F  S  A  L  S  E  G  A
__________________________________________HIV1 (IIIB) GAG/PRO GENE_________________________________________>

      2530      2540      2550      2560      2570      2580      2590      2600      2610      2620      2630      2640
ACCCCACAAGATTTAAACACCATGCTAAACACAGTGGGGGGACATCAAGCAGCCATGCAAATGTTAAAAGAGACCATCAATGAGGAAGCTGCAGAATGGGATAGAGTGCATCCAGTGCAT
TGGGGTGTTCTAAATTTGTGGTACGATTTGTGTCACCCCCCTGTAGTTCGTCGGTACGTTTACAATTTTCTCTGGTAGTTACTCCTTCGACGTCTTACCCTATCTCACGTAGGTCACGTA
 T  P  Q  D  L  N  T  M  L  N  T  V  G  G  H  Q  A  A  M  Q  M  L  K  E  T  I  N  E  E  A  A  E  W  D  R  V  H  P  V  H
__________________________________________HIV1 (IIIB) GAG/PRO GENE_________________________________________>

      2650      2660      2670      2680      2690      2700      2710      2720      2730      2740      2750      2760
GCAGGGCCTATTGCACCAGGCCAGATGAGAGAACCAAGGGGAAGTGACATAGCAGGAACTACTAGTACCCTTCAGGAACAAATAGGATGGATGACAAATAATCCACCTATCCCAGTAGGA
CGTCCCGGATAACGTGGTCCGGTCTACTCTCTTGGTTCCCCTTCACTGTATCGTCCTTGATGATCATGGGAAGTCCTTGTTTATCCTACCTACTGTTTATTAGGTGGATAGGGTCATCCT
 A  G  P  I  A  P  G  Q  M  R  E  P  R  G  S  D  I  A  G  T  T  S  T  L  Q  E  Q  I  G  W  M  T  N  N  P  P  I  P  V  G
__________________________________________HIV1 (IIIB) GAG/PRO GENE_________________________________________>

      2770      2780      2790      2800      2810      2820      2830      2840      2850      2860      2870      2880
GAAATTTATAAAAGATGGATAATCCTGGGATTAAATAAAATAGTAAGAATGTATAGCCCTACCAGCATTCTGGACATAAGACAAGGACCAAAAGAACCCTTTAGAGACTATGTAGACCGG
CTTTAAATATTTTCTACCTATTAGGACCCTAATTTATTTTATCATTCTTACATATCGGGATGGTCGTAAGACCTGTATTCTGTTCCTGGTTTTCTTGGGAAATCTCTGATACATCTGGCC
 E  I  Y  K  R  W  I  I  L  G  L  N  K  I  V  R  M  Y  S  P  T  S  I  L  D  I  R  Q  G  P  K  E  P  F  R  D  Y  V  D  R
__________________________________________HIV1 (IIIB) GAG/PRO GENE_________________________________________>

      2890      2900      2910      2920      2930      2940      2950      2960      2970      2980      2990      3000
TTCTATAAAACTCTAAGAGCCGAGCAAGCTTCACAGGAGGTAAAAAATTGGATGACAGAAACCTTGTTGGTCCAAAATGCGAACCCAGATTGTAAGACTATTTTAAAAGCATTGGGACCA
AAGATATTTTGAGATTCTCGGCTCGTTCGAAGTGTCCTCCATTTTTTAACCTACTGTCTTTGGAACAACCAGGTTTTACGCTTGGGTCTAACATTCTGATAAAATTTTCGTAACCCTGGT
 F  Y  K  T  L  R  A  E  Q  A  S  Q  E  V  K  N  W  M  T  E  T  L  L  V  Q  N  A  N  P  D  C  K  T  I  L  K  A  L  G  P
__________________________________________HIV1 (IIIB) GAG/PRO GENE_________________________________________>

      3010      3020      3030      3040      3050      3060      3070      3080      3090      3100      3110      3120
GCGGCTACACTAGAAGAAATGATGACAGCATGTCAGGGAGTAGGAGGACCCGGCCATAAGGCAAGAGTTTTGGCTGAAGCAATGAGCCAAGTAACAAATTCAGCTACCATAATGATGCAG
CGCCGATGTGATCTTCTTTACTACTGTCGTACAGTCCCTCATCCTCCTGGGCCGGTATTCCGTTCTCAAAACCGACTTCGTTACTCGGTTCATTGTTTAAGTCGATGGTATTACTACGTC
 A  A  T  L  E  E  M  M  T  A  C  Q  G  V  G  G  P  G  H  K  A  R  V  L  A  E  A  M  S  Q  V  T  N  S  A  T  I  M  M  Q
__________________________________________HIV1 (IIIB) GAG/PRO GENE_________________________________________>

      3130      3140      3150      3160      3170      3180      3190      3200      3210      3220      3230      3240
AGAGGCAATTTTAGGAACCAAAGAAAGATTGTTAAGTGTTTCAATTGTGGCAAAGAAGGGCACACAGCCAGAAATTGCAGGGCCCCTAGGAAAAAGGGCTGTTGGAAATGTGGAAAGGAA
TCTCCGTTAAAATCCTTGGTTTCTTTCTAACAATTCACAAAGTTAACACCGTTTCTTCCCGTGTGTCGGTCTTTAACGTCCCGGGGATCCTTTTTCCCGACAACCTTTACACCTTTCCTT
 R  G  N  F  R  N  Q  R  K  I  V  K  C  F  N  C  G  K  E  G  H  T  A  R  N  C  R  A  P  R  K  K  G  C  W  K  C  G  K  E
__________________________________________HIV1 (IIIB) GAG/PRO GENE_________________________________________>

      3250      3260      3270      3280      3290      3300      3310      3320      3330      3340      3350      3360
GGACACCAAATGAAAGATTGTACTGAGAGACAGGCTAATTTTTTAGGGAAGATCTGGCCTTCCTACAAGGGAAGGCCAGGGAATTTTCTTCAGAGCAGACCAGAGCCAACAGCCCCACCA
CCTGTGGTTTACTTTCTAACATGACTCTCTGTCCGATTAAAAAATCCCTTCTAGACCGGAAGGATGTTCCCTTCCGGTCCCTTAAAAGAAGTCTCGTCTGGTCTCGGTTGTCGGGGTGGT
 G  H  Q  M  K  D  C  T  E  R  Q  A  N  F  L  G  K  I  W  P  S  Y  K  G  R  P  G  N  F  L  Q  S  R  P  E  P  T  A  P  P
__________________________________________HIV1 (IIIB) GAG/PRO GENE_________________________________________>
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Fig. 7 (cont'd)

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    3370      3380      3390      3400      3410      3420      3430      3440      3450      3460      3470      3480
GAAGAGAGCTTCAGGTCTGGGGTAGAGACAACAACTCCCCCTCAGAAGCAGGAGCCGATAGACAAGGAACTGTATCCTTTAACTTCCCTCAGATCACTCTTTGGCAACGACCCCTCGTCA
CTTCTCTCGAAGTCCAGACCCCATCTCTGTTGTTGAGGGGGAGTCTTCGTCCTCGGCTATCTGTTCCTTGACATAGGAAATTGAAGGGAGTCTAGTGAGAAACCGTTGCTGGGGAGCAGT
E  E  S  F  R  S  G  V  E  T  T  T  P  P  Q  K  Q  E  P  I  D  K  E  L  Y  P  L  T  S  L  R  S  L  F  G  N  D  P  S  S
                                                                                            P  Q  I  T  L  W  Q  R  P  L  V
______________________________________________HIV1 (IIIB) GAG/PRO GENE____________________________________________________>

    3490      3500      3510      3520      3530      3540      3550      3560      3570      3580      3590      3600
AATAAAGATAGGGGGGCAACTAAAGGAAGCTCTATTAGATACAGGAGCAGATGATACAGTATTAGAAGAAATGAGTTTGCCAGGAAGATGGAAACCAAAAATGATAGGGGGAATTGGAG
TTATTTCTATCCCCCCGTTGATTTCCTTCGAGATAATCTATGTCCTCGTCTACTATGTCATAATCTTCTTTACTCAAACGGTCCTTCTACCTTTGGTTTTTACTATCCCCCTTAACCTC
Q
  I  K  I  G  G  Q  L  K  E  A  L  L  D  T  G  A  D  D  T  V  L  E  E  M  S  L  P  G  R  W  K  P  K  M  I  G  G  I  G
______________________________________________HIV1 (IIIB) GAG/PRO GENE____________________________________________________>

    3610      3620      3630      3640      3650      3660      3670      3680      3690      3700      3710      3720
TTTTATCAAAGTAAGACAGTATGATCAGATACTCATAGAAATCTGTGGACATAAAGCTATAGGTACAGTATTAGTAGGACCTACACCTGTCAACATAATTGGAAGAAATCTGTTGACTC
AAAATAGTTTCATTCTGTCATACTAGTCTATGAGTATCTTTAGACACCTGTATTTCGATATCCATGTCATAATCATCCTGGATGTGGACAGTTGTATTAACCTTCTTTAGACAACTGAG
 F  I  K  V  R  Q  Y  D  Q  I  L  I  E  I  C  G  H  K  A  I  G  T  V  L  V  G  P  T  P  V  N  I  I  G  R  N  L  L  T
______________________________________________HIV1 (IIIB) GAG/PRO GENE____________________________________________________>

    3730      3740      3750      3760      3770      3780      3790      3800
GATTGGTTGCACTTTAAATTTTTAACCCGGGGGATCCCGATTTTTATGACTAGTTAATCAAATAAAAAGCATACAAGCTATTGCTTC
CTAACCAACGTGAAATTTAAAAATTGGGCCCCCTAGGGCTAAAAATACTGATCAATTAGTTTATTTTTCGTATGTTCGATAACGAAG
 I  G  C  T  L  N  F          <____________________C3 FLANKING ARM____________________
_HIV1 (IIIB) GAG/PRO____>
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Fig. 8

vCP1452

K3L E3L in C6

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        10         20         30         40         50         60         70         80         90        100        110
GAGCTCGCGG CCGCCTATCA AAAGTCTTAA TGAGTTAGGT GTAGATAGTA TAGATATTAC TACAAAGGTA TTCATATTTC CTATCAATTC TAAAGTAGAT GATATTAATA
CTCGAGCGCC GGCGGATAGT TTTCAGAATT ACTCAATCCA CATCTATCAT ATCTATAATG ATGTTTCCAT AAGTATAAAG GATAGTTAAG ATTTCATCTA CTATAATTAT

       120        130        140        150        160        170        180        190        200        210        220
ACTCAAAGAT GATGATAGTA GATAATAGAT ACGCTCATAT AATGACTGCA AATTTGGACG GTTCACATTT TAATCATCAC GCGTTCATAA GTTTCAACTG CATAGATCAA
TGAGTTTCTA CTACTATCAT CTATTATCTA TGCGAGTATA TTACTGACGT TTAAACCTGC CAAGTGTAAA ATTAGTAGTG CGCAAGTATT CAAAGTTGAC GTATCTAGTT

       230        240        250        260        270        280        290        300        310        320        330
AATCTCACTA AAAAGATAGC CGATGTATTT GAGAGAGATT GGACATCTAA CTACGCTAAA GAAATTACAG TTATAAATAA TACATAATGG ATTTTGTTAT CATCAGTTAT
TTAGAGTGAT TTTTCTATCG GCTACATAAA CTCTCTCTAA CCTGTAGATT GATGCGATTT CTTTAATGTC AATATTTATT ATGTATTACC TAAAACAATA GTAGTCAATA

       340        350        360        370        380        390        400        410        420        430        440
ATTTAACATA AGTACAATAA AAAGTATTAA ATAAAAATAC TTACTTACGA AAAAATGACT AATTAGCTAT AAAAACCCAG ATCTCTCGAG GTCGACGGTA TCGATAAGCT
TAAATTGTAT TCATGTTATT TTTCATAATT TATTTTTATG AATGAATGCT TTTTTACTGA TTAATCGATA TTTTTGGGTC TAGAGAGCTC CAGCTGCCAT AGCTATTCGA

       450        460            470           480          490           500           510           520           530
TGATATCGAA TTCATAAAAA TT A TTG ATG TCT ACA CAT CCT TTT GTA ATT GAC ATC TAT ATA TCC TTT TGT ATA ATC AAC TCT AAT CAC TTT
ACTATAGCTT AAGTATTTTT AA T AAC TAC AGA TGT GTA GGA AAA CAT TAA CTG TAG ATA TAT AGG AAA ACA TAT TAG TTG AGA TTA GTG AAA
                           <Q   H   R   C   M   R   K   Y   N   V   D   I   Y   G   K   T   Y   D   V   R   I   V - K
                           ----------------------------------------k3L----------------------------------------------

      540         550          560           570           580           590           600           610           620
AAC TTT TAC AGT TTT CCC TAC CAG TTT ATC CCT ATA TTC AAC ATA TCT ATC CAT ATG CAT CTT AAC ACT CTC TGC CAA GAT AGC TTC AGA
TTG AAA ATG TCA AAA GGG ATG GTC AAA TAG GGA TAT AAG TTG TAT AGA TAG GTA TAC GTA GAA TTG TGA GAG ACG GTT CTA TCG AAG TCT
<V   K   V   T   K   G   V   L   K   D   R   Y   E   V   Y   R   D   M   H   M   K   V   S   E   A   L   I   A   E   S
-----------------------------------------------------------------K3L----------------------------------------------------

      630         640          650           660           670           680           690           700           710
GTG AGG ATA GTC AAA AAG ATA AAT GTA TAG AGC ATA ATC CTT CTC GTA TAC TCT GCC CTT TAT TAC ATC GCC CGC ATT GGG CAA CGA ATA
CAC TCC TAT CAG TTT TTC TAT TTA CAT ATC TCG TAT TAG GAA GAG CAT ATG AGA CGG GAA ATA ATG TAG CGG GCG TAA CCC GTT GCT TAT
<H   P   Y   D   F   L   Y   I   Y   L   A   Y   D   K   E   Y   V   R   G   K   I   V   D   G   A   N   P   L   S   Y
-----------------------------------------------------------------K3L----------------------------------------------------

      720         730        740        750        760        770        780        790        800        810
ACA AAA TGC AAG CAT ACG ATACAAACTT AACGGATATC GCGATAATGA AATAATTTAT GATTATTTCT CGCTTTCAAT TTAACACAAC CCTCAAGAAC
TGT TTT ACG TTC GTA TGC TATGTTTGAA TTGCCTATAG CGCTATTACT TTATTAAATA CTAATAAAGA GCGAAAGTTA AATTGTGTTG GGAGTTCTTG
<C   F   A   L   M
----------------K3L

       820        830        840        850        860        870        880        890        900        910        920
CTTTGTATTT ATTTTCACTT TTTAAGTATA GAATAAAGAA AGCTCTAATT AATTAATGAA CAGATTGTTT CGTTTTCCCC TTGGCGTATC ACTAATTAAT TAACCCGGGC
GAAACATAAA TAAAAGTGAA AAATTCATAT CTTATTTCTT TCGAGATTAA TTAATTACTT GTCTAACAAA GCAAAAGGGG AACCGCATAG TGATTAATTA ATTGGGCCCG

       930        940        950        960        970        980        990       1000       1010       1020       1030
TGCAGCTCGA GGAATTCAAC TATATCGACA TATTTCATTT GTATACACAT AACCATTACT AACGTAGAAT GTATAGGAAG AGATGTAACG GGAACAGGGT TTGTTGATTC
ACGTCGAGCT CCTTAAGTTG ATATAGCTGT ATAAAGTAAA CATATGTGTA TTGGTAATGA TTGCATCTTA CATATCCTTC TCTACATTGC CCTTGTCCCA AACAACTAAG

      1040       1050       1060       1070       1080       1090       1100       1110       1120       1130       1140
GCAAACTATT CTAATACATA ATTCTTCTGT TAATACGTCT TGCACGTAAT CTATTATAGA TGCCAAGATA TCTATATAAT TATTTTGTAA GATGATGTTA ACTATGTGAT
CGTTTGATAA GATTATGTAT TAAGAAGACA ATTATGCAGA ACGTGCATTA GATAATATCT ACGGTTCTAT AGATATATTA ATAAAACATT CTACTACAAT TGATACACTA
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Fig. 8 (cont'd)

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      1150       1160       1170       1180       1190       1200       1210       1220       1230       1240       1250
CTATATAAGT AGTGTAATAA TTCATGTATT TCGATATATG TTCCAACTCT GTCTTTGTGA TGTCTAGTTT CGTAATATCT ATAGCATCCT CAAAAAATAT ATTCGCATAT
GATATATTCA TCACATTATT AAGTACATAA AGCTATATAC AAGGTTGAGA CAGAAACACT ACAGATCAAA GCATTATAGA TATCGTAGGA GTTTTTTATA TAAGCGTATA

      1260       1270       1280       1290       1300       1310       1320       1330       1340       1350       1360
ATTCCCAAGT CTTCAGTTCT ATCTTCTAAA AAATCTTCAA CGTATGGAAT ATAATAATCT ATTTTACCTC TTCTGATATC ATTAATGATA TAGTTTTTGA CACTATCTTC
TAAGGGTTCA GAAGTCAAGA TAGAAGATTT TTTAGAAGTT GCATACCTTA TATTATTAGA TAAAATGGAG AAGACTATAG TAATTACTAT ATCAAAAACT GTGATAGAAG

      1370       1380       1390       1400       1410       1420       1430       1440       1450       1460       1470
TGTCAATTGA TTCTTATTCA CTATATCTAA GAAACGGATA GCGTCCCTAG GACGAACTAC TGCCATTAAT ATCTCTATTA TAGCTTCTGG ACATAATTCA TCTATTATAC
ACAGTTAACT AAGAATAAGT GATATAGATT CTTTGCCTAT CGCAGGGATC CTGCTTGATG ACGGTAATTA TAGAGATAAT ATCGAAGACC TGTATTAAGT AGATAATATG

      1480       1490       1500       1510       1520       1530       1540       1550       1560       1570       1580
CAGAATTAAT GGGAACTATT CCGTATCTAT CTAACATAGT TTTAAGAAAG TCAGAATCTA AGACCTGATG TTCATATATT GGTTCATACA TGAAATGATC TCTATTGATG
GTCTTAATTA CCCTTGATAA GGCATAGATA GATTGTATCA AAATTCTTTC AGTCTTAGAT TCTGGACTAC AAGTATATAA CCAAGTATGT ACTTTACTAG AGATAACTAC

      1590       1600       1610       1620       1630       1640       1650       1660       1670       1680       1690
ATAGTGACTA TTTCATTCTC TGAAAATTGG TAACTCATTC TATATATGCT TTCCTTGTTG ATGAAGGATA GAATATACTC AATAGAATTT GTACCAACAA ACTGTTCTCT
TATCACTGAT AAAGTAAGAG ACTTTTAACC ATTGAGTAAG ATATATACGA AAGGAACAAC TACTTCCTAT CTTATATGAG TTATCTTAAA CATGGTTGTT TGACAAGAGA

      1700       1710       1720       1730       1740       1750       1760       1770       1780       1790       1800
TATGAATCGT ATATCATCAT CTGAAATAAT CATGTAAGGC ATACATTTAA CAATTAGAGA CTTGTCTCCT GTTATCAATA TACTATTCTT GTGATAATTT ATGTGTGAGG
ATACTTAGCA TATAGTAGTA GACTTTATTA GTACATTCCG TATGTAAATT GTTAATCTCT GAACAGAGGA CAATAGTTAT ATGATAAGAA CACTATTAAA TACACACTCC

      1810       1820       1830       1840       1850       1860       1870       1880       1890       1900       1910
CAAATTTGTC CACGTTCTTT AATTTTGTTA TAGTAGATAT CAAATCCAAT GGAGCTACAG TTCTTGGCTT AAACAGATAT AGTTTTTCTG GAACAAATTC TACAACATTA
GTTTAAACAG GTGCAAGAAA TTAAAACAAT ATCATCTATA GTTTAGGTTA CCTCGATGTC AAGAACCGAA TTTGTCTATA TCAAAAAGAC CTTGTTTAAG ATGTTGTAAT

      1920       1930       1940       1950       1960       1970       1980       1990       2000       2010       2020
TTATAAAGGA CTTTGGGTAG ATAAGTGGGA TGAAATCCTA TTTTAATTAA TGCTATCGCA TTGTCCTCGT GCAAATATCC AAACGCTTTT GTGATAGTAT GGCATTCATT
AATATTTCCT GAAACCCATC TATTCACCCT ACTTTAGGAT AAAATTAATT ACGATAGCGT AACAGGAGCA CGTTTATAGG TTTGCGAAAA CACTATCATA CCGTAAGTAA

      2030       2040       2050       2060       2070       2080       2090       2100       2110       2120       2130
GTCTAGAAAC GCTCTACGAA TATCTGTGAC AGATATCATC TTTAGAGAAT ATACTAGTCG CGTTAATAGT ACTACAATTT GTATTTTTTA ATCTATCTCA ATAAAAAAAT
CAGATCTTTG CGAGATGCTT ATAGACACTG TCTATAGTAG AAATCTCTTA TATGATCAGC GCAATTATCA TGATGTTAAA CATAAAAAAT TAGATAGAGT TATTTTTTTA

      2140       2150       2160       2170       2180       2190           2200           2210           2220           2230
TAATATGTAT GATTCAATGT ATAACTAAAC TACTAACTGT TATTGATAAC TAGAATCA GAA TCT AAT GAT GAC GTA ACC AAG AAG TTT ATC TAC TGC CAA
ATTATACATA CTAAGTTACA TATTGATTTG ATGATTGACA ATAACTATTG ATCTTAGT CTT AGA TTA CTA CTG CAT TGG TTC TTC AAA TAG ATG ACG GTT
                                                                <F   R   I   I   V   Y   G   L   L   K   D   V   A   L
                                                                -------E3L---------------------------------------------

        2240           2250           2260           2270           2280           2290           2300           2310           2320
TTT AGC TGC ATT ATT TTT AGC ATC TCG TTT AGA TTT TCC ATC TGC CTT ATC GAA TAC TCT TCC GTC GAT GTC TAC ACA GGC ATA AAA TGT
AAA TCG ACG TAA TAA AAA TCG TAG AGC AAA TCT AAA AGG TAG ACG GAA TAG CTT ATG AGA AGG CAG CTA CAG ATG TGT CCG TAT TTT ACA
<K   A   A   N   N   K   A   D   R   K   S   K   G   D   A   K   D   F   V   R   G   D   I   D   V   C   A   Y   F   T
-------------------------------------------------------------E3L---------------------------------------------------------
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Fig. 8 (cont'd)

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2330      2340      2350      2360      2370      2380      2390      2400      2410
AGG AGA GTT ACT AGG CCC AAC TGA TTC AAT ACG AAA AGA CCA ATC TCT CTT AGT TAT TTG GCA GTA CTC ATT AAT AAT GGT GAC AGG GTT
TCC TCT CAA TGA TCC GGG TTG ACT AAG TTA TGC TTT TCT GGT TAG AGA GAA TCA ATA AAC CGT CAT GAG TAA TTA TTA CCA CTG TCC CAA
<P  S   N   S   P   G   V   S   E   I   R   F   S   W   D   R   K   T   I   Q   C   Y   E   N   I   I   T   V   P   N
-------------------------------------------------------E3L--------------------------------------------------------

     2420      2430      2440      2450      2460      2470      2480      2490      2500
AGC ATC TTT CCA ATC AAT AAT TTT TTT AGC CGG AAT AAC ATC ATC AAA AGA CTT ATG ATC CTC TCT CAT TGA TTT TTC GCG GGA TAC ATC
TCG TAG AAA GGT TAG TTA TTA AAA AAA TCG GCC TTA TTG TAG TAG TTT TCT GAA TAC TAG GAG AGA GTA ACT AAA AAG CGC CCT ATG TAG
<A  D   K   W   D   I   I   K   K   A   P   I   V   D   D   F   S   K   H   D   E   R   M   S   K   E   R   S   V   D
-------------------------------------------------------E3L--------------------------------------------------------

     2510      2520      2530      2540      2550      2560      2570      2580      2590
ATC TAT TAT GAC GTC AGC CAT AGC ATC AGC ATC CGG CTT ATC CGC CTC CGT TGT CAT AAA CCA ACG AGG AGG AAT ATC GTC GGA GCT GTA
TAG ATA ATA CTG CAG TCG GTA TCG TAG TCG TAG GCC GAA TAG GCG GAG GCA ACA GTA TTT GGT TGC TCC TCC TTA TAG CAG CCT CGA CAT
<D  I   I   V   D   A   M   A   D   A   D   P   K   D   A   E   T   T   M   F   W   R   P   P   I   D   D   S   S   Y
-------------------------------------------------------E3L--------------------------------------------------------

     2600      2610      2620      2630      2640      2650      2660      2670      2680
CAC CAT AGC ACT ACG TTG AAG ATC GTA CAG AGC TTT ATT AAC TTC TCG CTT CTC CAT ATT AAG TTG TCT AGT TAG TTG TGC AGC AGT AGC
GTG GTA TCG TGA TGC AAC TTC TAG CAT GTC TCG AAA TAA TTG AAG AGC GAA GAG GTA TAA TTC AAC AGA TCA ATC AAC ACG TCG TCA TCG
<V  M   A   S   R   Q   L   D   Y   L   A   K   N   V   E   R   K   E   M   N   L   Q   R   T   L   Q   A   A   T   A
-------------------------------------------------------E3L--------------------------------------------------------

     2690      2700      2710      2720      2730      2740      2750      2760      2770
TCC TTC GAT TCC AAT GTT TTT AAT AGC CGC ACA CAC AAT CTC TGC GTC AGA ACG CTC GTC AAT ATA GAT CTT AGA CAT TT TTAGAGAGAA
AGG AAG CTA AGG TTA CAA AAA TTA TCG GCG TGT GTG TTA GAG ACG CAG TCT TGC GAG CAG TTA TAT CTA GAA TCT GTA AA AATCTCTCTT
<G  E   I   G   I   N   K   I   A   A   C   V   I   E   A   D   S   R   E   D   I   Y   I   K   S   M
-------------------------------------------------------E3L--------------------------

      2780       2790       2800       2810       2820       2830       2840       2850       2860       2870       2880
CTAACACAAC CAGCAATAAA ACTGAACCTA CTTTATCATT TTTTTATTCA TCATCCTCTG GTGGTTCGTC GTTTCTATCG AATGTAGCTC TGATTAACCC GTCATCTATA
GATTGTGTTG GTCGTTATTT TGACTTGGAT GAAATAGTAA AAAAATAAGT AGTAGGAGAC CACCAACCAG CAAAGATAGC TTACATCGAG ACTAATTGGG CAGTAGATAT

      2890       2900       2910       2920       2930       2940       2950       2960       2970       2980       2990
GGTGATGCTG GTTCTGGAGA TTCTGGAGGA GATGGATTAT TATCTGGAAG AATCTCTGTT ATTTCCTTGT TTTCATGTAT CGATTGCGTT GTAACATTAA GATTGCGAAA
CCACTACGAC CAAGACCTCT AAGACCTCCT CTACCTAATA ATAGACCTTC TTAGAGACAA TAAAGGAACA AAAGTACATA GCTAACGCAA CATTGTAATT CTAACGCTTT

      3000       3010       3020       3030       3040       3050       3060       3070       3080       3090       3100
TGCTCTAAAT TTGGGAGGCT TAAAGTGTTG TTTGCAATCT CTACACGCGT GTCTAACTAG TGGAGGTTCG TCAGCTGCTC TAGTTTGAAT CATCATCGGC GTAGTATTCC
ACGAGATTTA AACCCTCCGA ATTTCACAAC AAACGTTAGA GATGTGCGCA CAGATTGATC ACCTCCAAGC AGTCGACGAG ATCAAACTTA GTAGTAGCCG CATCATAAGG

      3110       3120       3130       3140       3150       3160       3170       3180       3190       3200       3210
TACTTTTACA GTTAGGACAC GGTGTATTGT ATTTCTCGTC GAGAACGTTA AAATAATCGT TGTAACTCAC ATCCTTTATT TTATCTATAT TGTATTCTAC TCCTTTCTTA
ATGAAAATGT CAATCCTGTG CCACATAACA TAAAGAGCAG CTCTTGCAAT TTTATTAGCA ACATTGAGTG TAGGAAATAA AATAGATATA ACATAAGATG AGGAAAGAAT

      3220       3230       3240       3250       3260       3270       3280       3290       3300       3310       3320
ATGCATTTTA TACCGAATAA GAGATAGCGA AGGAATTCTT TTTATTGATT AACTAGTCAA ATGAGTATAT ATAATTGAAA AAGTAAAATA TAAATCATAT AATAATGAAA
TACGTAAAAT ATGGCTTATT CTCTATCGCT TCCTTAAGAA AAATAACTAA TTGATCAGTT TACTCATATA TATTAACTTT TTCATTTTAT ATTTAGTATA TTATTACTTT
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Fig. 8 (cont'd)

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      3330       3340       3350       3360       3370       3380       3390       3400       3410       3420       3430
   *    *     *    *     *    *     *    *     *    *     *    *     *    *     *    *     *    *     *    *     *    *
CGAAATATCA GTAATAGACA GGAACTGGCA GATTCTTCTT CTAATGAAGT AAGTACTGCT AAATCTCCAA AATTAGATAA AAATGATACA GCAAATACAG CTTCATTCAA
GCTTTATAGT CATTATCTGT CCTTGACCGT CTAAGAAGAA GATTACTTCA TTCATGACGA TTTAGAGGTT TTAATCTATT TTTACTATGT CGTTTATGTC GAAGTAAGTT

      3440       3450       3460       3470       3480       3490       3500       3510       3520       3530       3540
   *    *     *    *     *    *     *    *     *    *     *    *     *    *     *    *     *    *     *    *     *    *
CGAATTACCT TTTAATTTTT TCAGACACAC CTTATTACAA ACTAACTAAG TCAGATGATG AGAAAGTAAA TATAAATTTA ACTTATGGGT ATAATATAAT AAAGATTCAT
GCTTAATGGA AAATTAAAAA AGTCTGTGTG GAATAATGTT TGATTGATTC AGTCTACTAC TCTTTCATTT ATATTTAAAT TGAATACCCA TATTATATTA TTTCTAAGTA

      3550       3560       3570       3580       3590       3600       3610       3620       3630       3640       3650
   *    *     *    *     *    *     *    *     *    *     *    *     *    *     *    *     *    *     *    *     *    *
GATATTAATA ATTTACTTAA CGATGTTAAT AGACTTATTC CATCAACCCC TTCAAACCTT TCTGGATATT ATAAAATACC AGTTAATGAT ATTAAAATAG ATTGTTTAAG
CTATAATTAT TAAATGAATT GCTACAATTA TCTGAATAAG GTAGTTGGGG AAGTTTGGAA AGACCTATAA TATTTTATGG TCAATTACTA TAATTTTATC TAACAAATTC

      3660       3670       3680       3690       3700       3710       3720       3730       3740       3750       3760
   *    *     *    *     *    *     *    *     *    *     *    *     *    *     *    *     *    *     *    *     *    *
AGATGTAAAT AATTATTTGG AGGTAAAGGA TATAAAATTA GTCTATCTTT CACATGGAAA TGAATTACCT AATATTAATA ATTATGATAG GAATTTTTTA GGATTTACAG
TCTACATTTA TTAATAAACC TCCATTTCCT ATATTTTAAT CAGATAGAAA GTGTACCTTT ACTTAATGGA TTATAATTAT TAATACTATC CTTAAAAAAT CCTAAATGTC

      3770       3780       3790       3800       3810       3820       3830       3840       3850       3860       3870
   *    *     *    *     *    *     *    *     *    *     *    *     *    *     *    *     *    *     *    *     *    *
CTGTTATATG TATCAACAAT ACAGGCAGAT CTATGGTTAT GGTAAAACAC TGTAACGGGA AGCAGCATTC TATGGTAACT GGCCTATGTT TAATAGCCAG ATCATTTTAC
GACAATATAC ATAGTTGTTA TGTCCGTCTA GATACCAATA CCATTTTGTG ACATTGCCCT TCGTCGTAAG ATACCATTGA CCGGATACAA ATTATCGGTC TAGTAAAATG

      3880       3890       3900       3910       3920       3930       3940       3950       3960       3970       3980
   *    *     *    *     *    *     *    *     *    *     *    *     *    *     *    *     *    *     *    *     *    *
TCTATAAACA TTTTACCACA AATAATAGGA TCCTCTAGAT ATTTAATATT ATATCTAACA ACAACAAAAA AATTTAACGA TGTATGGCCA GAAGTATTTT CTACTAATAA
AGATATTTGT AAAATGGTGT TTATTATCCT AGGAGATCTA TAAATTATAA TATAGATTGT TGTTGTTTTT TTAAATTGCT ACATACCGGT CTTCATAAAA GATGATTATT

      3990       4000       4010       4020       4030       4040       4050       4060       4070       4080       4090
   *    *     *    *     *    *     *    *     *    *     *    *     *    *     *    *     *    *     *    *     *    *
AGATAAAGAT AGTCTATCTT ATCTACAAGA TATGAAAGAA GATAATCATT TAGTAGTAGC TACTAATATG GAAAGAAATG TATACAAAAA CGTGGAAGCT TTTATATTAA
TCTATTTCTA TCAGATAGAA TAGATGTTCT ATACTTTCTT CTATTAGTAA ATCATCATCG ATGATTATAC CTTTCTTTAC ATATGTTTTT GCACCTTCGA AAATATAATT

      4100       4110       4120       4130       4140       4150       4160       4170       4180       4190       4200
   *    *     *    *     *    *     *    *     *    *     *    *     *    *     *    *     *    *     *    *     *    *
ATAGCATATT ACTAGAAGAT TTAAAATCTA GACTTAGTAT AACAAAACAG TTAAATGCCA ATATCGATTC TATATTTCAT CATAACAGTA GTACATTAAT CAGTGATATA
TATCGTATAA TGATCTTCTA AATTTTAGAT CTGAATCATA TTGTTTTGTC AATTTACGGT TATAGCTAAG ATATAAAGTA GTATTGTCAT CATGTAATTA GTCACTATAT

      4210       4220       4230       4240       4250       4260       4270       4280       4290       4300       4310
   *    *     *    *     *    *     *    *     *    *     *    *     *    *     *    *     *    *     *    *     *    *
CTGAAACGAT CTACAGACTC AACTATGCAA GGAATAAGCA ATATGCCAAT TATGTCTAAT ATTTTAACTT TAGAACTAAA ACGTTCTACC AATACTAAAA ATAGGATACG
GACTTTGCTA GATGTCTGAG TTGATACGTT CCTTATTCGT TATACGGTTA ATACAGATTA TAAAATTGAA ATCTTGATTT TGCAAGATGG TTATGATTTT TATCCTATGC

      4320       4330       4340       4350       4360       4370       4380       4390       4400       4410       4420
   *    *     *    *     *    *     *    *     *    *     *    *     *    *     *    *     *    *     *    *     *    *
TGATAGGCTG TTAAAAGCTG CAATAAATAG TAAGGATGTA GAAGAAATAC TTTGTTCTAT ACCTTCGGAG GAAAGAACTT TAGAACAACT TAAGTTTAAT CAAACTTGTA
ACTATCCGAC AATTTTCGAC GTTATTTATC ATTCCTACAT CTTCTTTATG AAACAAGATA TGGAAGCCTC CTTTCTTGAA ATCTTGTTGA ATTCAAATTA GTTTGAACAT

      4430
   *    *
TTTATGAAGG TACC
AAATACTTCC ATGG
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INTERNATIONAL SEARCH REPORT

International application No.
PCT/US98/02669

A. CLASSIFICATION OF SUBJECT MATTER

IPC(6) :C12N 15/64, 15/67, 15/86; A61K 48/00
US CL :424/93.2, 199.1; 435/69.1, 172.3, 320.1
According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 424/93.2, 199.1; 435/69.1, 172.3, 320.1

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

APS; DIALOG DATABASES: MEDLINE, BIOSIS, CA SEARCH, WORLD PATENT INDEX

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	US 4,738,922 A (HASELTINE et al.) 19 April 1988, see entire document.	1-14, 18-26
A	PARK, H. et al. TAR RNA-binding protein is an inhibitor of the interferon-induced protein kinase PKR. Proc. Natl. Acad. Sci. USA. May 1994, Vol. 91, pages 4713-4717. See entire article.	1-14, 18-26

☐ Further documents are listed in the continuation of Box C. ☐ See patent family annex.

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"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
02 APRIL 1998	20 JUL 1998

Name and mailing address of the ISA/US	Authorized officer
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